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THE SPECIES CONCEPT FROM THE POINT OF VIEW OF A GENETICIST[1]

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Classification is a fundamental characteristic of scientific work in every field, and recognition of the fact that plants and animals may be described and discussed in group terms was doubtless the earliest manifestation of biological science. The advantages to every branch of biology of having group descriptions designated by brief names, which pass current in the language of all civilized peoples, is so manifest as to make the statement of the fact little more than a platitude. Indeed, the grouping of individuals into definitely limited categories whose names are common to all languages may be likened in its importance to the discovery of language itself. It provides the means of intercommunication, not only among biologists, but between biologists and humanity in general.

In order that this primary function of biological classification, as a basis of effective intercommunication, may be fulfilled, it is important that the limitations of the categories be such as may be capable of observation and comprehension by those among whom intercommunication is to be established or maintained. It seems, therefore, that the proper basis for classification is intimately related to the question of convenience. A consideration of all the different grades of classification: kingdoms, phyla, orders, families, genera, species, subspecies, forms, etc., etc., shows that the *species* represents the simplest concept, and it is therefore the one best adapted to serve as a vehicle of *general* communication.

Species may be defined as the easily recognized kinds of organisms, and in the case of macroscopic plants and animals their recognition should rest on simple gross observation such as any intelligent person can make with the aid only, let us say, of a good hand-lens. Genera, families, and higher orders of classification belong rather to biological philosophy, while the subspecific categories represent refinements which are of interest only or chiefly to specialists. In other words, *species* belong to biological "Main Street," and their chief usefulness lies in the very fact of the ease and definiteness with which they may be recognized.

[1] Read in the symposium on "The Utility of the Species Concept," at the joint meeting of Section G of the American Association for the Advancement of Science, the American Phytopathological Society, and the Botanical Society of America, at Toronto, December 28, 1921.

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The geneticist has a peculiar interest in species and has always dealt with problems which have to do with the real inwardness of the species concept. Biological philosophers have often given genetical definitions of the species concept, as did the "Father of Botany" himself in a certain limited sense when he assumed that there are "as many species as the Infinite Being originally produced different forms."

The first great geneticist, J. G. Koelreuter, devoted a great deal of attention to an attempt to determine whether certain putative species were in reality specifically distinct or only varietally, basing his conclusions on the degree of sterility or fertility of the two forms under test, when bred together, and upon the fertility or sterility of the hybrids produced by such cross-breeding.

Not only this capacity for normal interbreeding, but also the degree of variability which occurs within normally interbreeding groups, have served as useful genetical characteristics of specific delimitation; but the usefulness of these phenomena as aids in determining what groups to call species is greatly lessened by the facts that so many species do not normally interbreed, and that not a few can not interbreed, as, for example, the parthenogenetic species of Taraxacum, Antennaria, etc.

These facts make it necessary, in the use of such a criterion of species, *merely to estimate* the amount of variation *which might be expected* to occur normally within a freely interbreeding group; and when we must resort to this sort of abstraction, the method at once loses its value in relation to the discovery of *natural* limitations of species. The fact is that, although genetical phenomena form the basis of nearly all biological classification, there is no *genetical* criterion—*nor any other criterion*—of specific difference, which is found generally applicable or generally acceptable. The geneticist comes more and more to the point of view that the distinctions between species are only quasi-natural, that specific differences are by no means quantitatively equivalent in different genera, families, orders, or phyla, but that genetically there are very many different grades of distinction between the form-groups which are, with more or less justification, recognized by taxonomists as "species."

This being the case, it seems proper to insist that utilitarian principles should be crucial in the establishment of new species and the maintenance of old ones. When we consider the whole question from the standpoint of convenience, it is clear that the needs and experiences of the user must be determinative.

To the systematist, whether professional or amateur, the species classification is the "bread of life"; but to other classes of biologists the species is merely a tool, handy or unhandy according as the taxonomist has done his work wisely or unwisely. To these other classes of biologists the species exist for the biologist, not the biologist for the species. Hence it often happens that the physiologist, the ecologist, the geneticist, etc., take

what doubtless seem to the taxonomist rather shocking liberties with the taxonomic species.

A simple example from my own work will illustrate how taxonomic distinctions may become useless to the geneticist. For a number of years I bred the dioecious forms of Lychnis (Melandrium) quite extensively, growing many hundreds of pedigreed families from seeds secured from various parts of Europe and America. I found that nearly every lot of seeds produced a progeny perceptibly different from those produced from other lots of seeds; but the differences, which were in some cases sharp and easily stated in precise terms, were in other cases difficult of exact description. All these forms—of whatever name and wherever collected—bred together without any diminution in fertility—in fact, usually with increased fecundity; and, although there were numerous differentiating hereditary characters, these were not grouped in the wild forms in such manner as to make it profitable or convenient to attempt to keep track of specific distinctions, as such, in the experimental garden. In my published accounts of these experiments the Linnaean designation, *Lychnis dioica*, was the only one which could be usefully employed, the English forms *L. diurna* and *L. vespertina* and the German forms *Melandrium album* and *M. rubrum* being incapable of maintenance.

Since the condition which here made the minor distinctions between taxonomic species useless was the perfect freedom with which all forms interbred, and the independence with which the hereditary characteristics were distributed, Koelreuter's criterion of specific difference receives support, and it might be assumed that under experimental conditions Koelreuter's method of delimiting species on the basis of compatibility or incompatibility —fertility *vs.* sterility—would have *general* genetical validity, except in the cases already mentioned in which vegetative or parthenogenetic methods of breeding occur; but any attempt to make extensive use of this idea promptly meets difficulties of most formidable dimensions on account of the numerous kinds and degrees of incompatibility and sterility which are met with.

Sterility may result from the presence of a single unit factor, of no more fundamental nature than the factor which changes a white flower to a colored one, or *vice versa*, in any one of a large number of species which might be mentioned. In such a case only a single gene differentiates the two forms, all the rest of the complex organization of the genotype being identical. On the other hand, incompatibility and sterility may result from general dissimilarities in organization, involving, conceivably, innumerable details of genotypic structure. I may cite several examples from my experiments with Bursa to illustrate the difficulties attendant upon any attempt to utilize incompatibilities and sterilities as *general criteria* of specific differences.

For fifteen years I have been working extensively with the common

shepherd's purse (*Bursa bursa-pastoris*) and to a less extent with several other species of the same genus. In connection with this work I have had material sent to me from nearly every part of the temperate regions of the world. Every new lot of material received has added to the demonstration that there occurs in nature an amazing number of hereditarily distinct forms within the species *B. bursa-pastoris*. While these biotypes are genetical entities of perfect delimitation, they can not by any method be given taxonomic validity as species or subspecies, because, under natural conditions, the genotypic factors prove to be less effective in determining the visible qualities which are necessarily utilized in classification by inspection than are the environmental factors. Even the larger features of leaf-lobing to which I have assigned the subspecific names *heteris*, *rhomboidea*, *tenuis*, and *simplex* are, oftener than not, quite indistinguishable in the field, and it has not been infrequent to have forms sent to me by good taxonomists, under the name "*integrifolia*," turn out to be *heteris*, the most highly lobed type. The significance of this is that the distinctive *genetical* characters are easily suppressed by crowding, poor soil, poor illumination, and other adverse conditions. The only practicable thing to do in a case of this kind is to maintain the species *bursa-pastoris*, recognizing its composite character, and in so doing to recognize also that species must be based upon characters whose existence is not too seriously affected by deviations in the environment.

In relation to the question of sterility of hybrids as evidence of specific distinctness in the parents, I may cite the following cases in *Bursa bursa-pastoris*, and its hybrids with *B. grandiflora* and *B. Viguieri*.

In 1913 I received from Professor A. H. Trow, of Cardiff, Wales, several packets of seed of Bursa specimens growing in close proximity to one another at Cardiff. Two of the progenies grown from these seeds differed slightly in outline of the capsules and in form and surface of the leaves, but neither suggested by its appearance that it ought to serve as the type of a newly defined species, and, indeed, it is doubtful whether they could have been separated at all when growing in nature. However, the two strains thus derived, when crossed together, gave hybrids which were almost completely sterile. When two types are thus found intermingled in nature and not capable of ready separation on inspection, they can not properly be referred to distinct species, even though their crosses show sterility; but, on the other hand, when we find that all up and down the Pacific coast of North and South America and extending eastward at least as far as Tucson, Arizona, and Waco, Texas, a characteristic form of Bursa occurs, having invariably concave-sided capsules, and yielding only sterile or almost sterile hybrids when crossed with numerous biotypes of *B. bursa-pastoris* from Europe and the eastern half of North America, which latter biotypes have prevailingly convex-sided capsules, one may with considerable justification urge the recognition of this west-American form as a species.

distinct from the species common to eastern America and Europe. In other words, it is *convenient* to differentiate between groups of organisms occupying different geographical regions, even though the differentiating characteristics are few and of relatively insignificant magnitude, while it is impracticable to separate *intermingled* groups differentiated by similar relatively slight characters.

A second kind of sterility is exhibited within the very distinct large-flowered species, *Bursa grandiflora* Bois., native of the regions about the Aegean Sea. In this species I have been able to demonstrate that there are two groups of plants, A and B, not visibly differentiable, both of which are sterile or nearly so when selfed or when crossed with other individuals of their own kind, but which are fully fertile when any two individuals mated together belong to different groups. In other words, $A \times$ self or $A \times A$, and $B \times$ self or $B \times B$, yield no offspring or almost none, while $A \times B$ or $B \times A$ is fully fertile and produces seeds abundantly. Here we are dealing with so-called self-sterility and cross-fertility; but, as already stated, there is also *cross-sterility* here, since A crossed with any other A plant or B with any other B plant produces no seeds. No one can maintain that incompatible crosses in such a case as this indicate specific distinctness between the parents, for *it is just the most closely related individuals*, those of like genotypic constitution, that are incompatible.

When we consider the results of crossing together such very distinct species as *Bursa bursa-pastoris*, *B. grandiflora*, and *B. Viguieri*, an even more striking limitation on hybrid sterility as an indication of specific distinctness is impressed. The wide differences in the three species here named may be noted in table 1, in which are entered their several contrasting characters.

TABLE 1

	Grandiflora	*Bursa-pastoris*	*Viguieri*
Rosettes	Lax	Rather lax	Dense
Leaves	Always well lobed	Variously lobed	But little lobed
Surface	Smooth, shining	Moderately smooth	Rugose, dull
Stellate hairs	Numerous	Abundant	Rare, small
Stems	Normal	Normal	Fasciated
Flowers	Large	Medium size	Small
Odor	Strong balsam-like	Absent	Absent
Breeding	Self-sterile	Self-fertile	Self-fertile
Carpels	2	2	3 to 8 (mode 4)

During the past six years I have made more than twenty hybridizations between *B. Viguieri* and *B. bursa-pastoris*, using in each case a different biotype of the latter species. Hybrids have always been easily secured from these crosses, the fecundity being at least approximately as great as when pollen from the same species has been used. The hybrids have always been vigorous plants of the *bursa-pastoris* type, no matter which

species played the rôle of mother in the cross, but these hybrids were always so nearly sterile that in no case was more than one seed found in any capsule of one of the F_1 plants, nor were more than two capsules, each with one seed, ever found on any one such plant, and only rarely did an F_1 plant have even one seed. At one time two F_2 plants were secured from these rare seeds, but these were lost before their adult characters had been observed. The remarkable distinctness of the two species, *bursa-pastoris* and *Viguieri*, left no ground for surprise at this F_1 sterility, and one would be inclined, perhaps, to say that *Viguieri* has deviated so far from the *bursa-pastoris* type, from which it doubtless took its origin, that it can no longer produce fertile progenies when back-crossed to the ancestral type.

When crosses are made between *B. bursa-pastoris* and *B. grandiflora*, a somewhat different relationship is indicated. Nearly twenty crosses of this type have also been made, but only in one case have F_1 hybrids (3) been secured, though the pollination usually results in the full development of the capsules and the ovules usually enlarge to nearly full size and then abort. Of three hybrids secured from one of these crosses, all were partially fertile, but fertility was slight in two, and in the third it was far from complete. From the most highly fertile of these an F_2 was grown which was so nearly sterile that it was not considered practicable to pursue the experiment farther.

The complete failure to secure F_1 hybrids from all but this one cross is in marked contrast with the easy production of F_1 hybrids in the *bursa-pastoris-Viguieri* crosses. Here again it may seem a logical explanation to say that *Bursa grandiflora* has evolved so many (or such considerable) divergences from the *bursa-pastoris* type that it can only rarely produce hybrids when crossed to the latter, thus giving seemingly good ground for the acceptance of such hybrid sterility as a criterion of specific distinctness.

But what becomes of the validity of this criterion when it is found that *Bursa grandiflora* and *B. Viguieri*, which give only sterile hybrids, or none at all, with the intermediately placed *B. bursa-pastoris*, *produce fully fertile hybrids when crossed with each other?* As seen from the table given above, the difference between *B. grandiflora* and *B. Viguieri* is essentially the sum of the differences of each from *B. bursa-pastoris*, so that we can not logically assume that they are specifically distinct from the latter species and only subspecifically distinct from each other.

The non-validity of the sterility criterion is already fully recognized by every one in the case of the Orchidaceae, where fully fertile hybrids are produced, not only between very distinct species, but also between many different genera.

Still another case in Bursa may be cited to show that two forms recognized as species by taxonomists may differ in only a relatively simple genotypic feature which does not affect capacity for interbreeding. *Bursa Heegeri* (Solms-Laubach), which was found by Professor Heeger on the market-place at Landau, Germany, in 1890, is so distinct from *B. bursa-*

pastoris that Solms-Laubach, to whom it was sent for identification, was inclined to place it in the genus Camelina, because of its rounded, uninflated capsules. Only when he observed a mutation (*Bursa Solmsiana* Shull *ined.*) arising from it, which exhibited a partial reversion to the *bursa-pastoris* type of capsule, did he recognize the identity of the new form as a species of Bursa.

I have made a very large number of hybridizations between *B. Heegeri* and different biotypes of *B. bursa-pastoris*, and these two species have bred together with perfect readiness and with full fertility, so long as the biotypes of *bursa-pastoris* used were of European or east-American origin. The segregation showed that a simple Mendelian difference exists between these two species, though this difference is quite generally duplicated through the presence of the corresponding factor or gene in two different chromosome pairs. It is thus seen that genotypically the species-character which differentiates *B. Heegeri* from *B. bursa-pastoris* is on an exact par with the character of leaf-lobing which differentiates the several rosette types of Bursa into those which have the sinuses extending to the midrib (*heteris* and *rhomboidea*) and those which have the sinuses less deep (*tenuis* and *simplex*), which differentials, as we have already seen, can not be used as satisfactory characters in taxonomy because they are too easily suppressed by environmental factors. It is obvious, therefore, that the capsule form is here a satisfactory specific character because it is unmodified by variations in the environment, and because it is therefore always available for use in determining the identity of any individual, while the *genotypically equal* differences in leaf-lobing are not suitable specific characters because they lack this quality of being invariably expressed when the genotypic constellation requisite for their expression is present.

Since there is no natural hiatus in the range of visibility of different phenotypic characters possessed by different individuals or groups of plants and animals, nor in the degree of their persistence under the normal variations of the environment, I believe that my statement, made above, is substantiated, that *species are only quasi-natural entities* and that they are made so by the lack of agreement between external appearance and internal constitution and by the low visibility of many hereditary characteristics. Natural groups there certainly are, but these are the biotypes of the geneticist, not the species of the taxonomist. Only here and there is there a coincidence between biotype and species.

Since the usefulness of the species concept rests upon the exchange value of specific names in scientific and intellectual intercourse, it must be borne in mind that an undue increase in the number of species has the same effect on the exchange value of the species in the intellectual markets of the world that analogous inflation of financial currency has upon the value of any monetary unit involved in such inflation. Compare, for example, the usefulness of Crataegus species today with the decline in value of the

franc and the lira, if not perhaps with that of the mark and the ruble, and let the taxonomist take warning not to destroy the usefulness of the species concept to biology in general by lowering the degree of visibility of the characteristics which delimit adjacent categories. For the minor groups which his more intensive studies may bring to light let him adopt a special terminology adequate to meet his needs in communicating with other taxonomic specialists, just as the geneticists have done. It is to be hoped, however, that the taxonomist will not find it necessary to propose such a profusion of names for these subspecific categories as have the geneticists. Witness: "elementary species," "biotype," "Jordanon," "isoreagent," "genospecies," "microspecies," "microgene," etc.

If all biological specialists, in whatever direction their specialties may lie, should adopt this method, species will continue to represent the relatively crude, relatively superficial triangulation of the entire field of biology by means solely of such instrumentation as is available, in common, to the devotees of every branch of the science, and the specific names will retain their high value as media of scientific exchange.

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THE SPECIES CONCEPT FROM THE POINT OF VIEW OF A MORPHOLOGIST[1]

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On the reality of organic evolution we all agree; but has the species concept outlived its usefulness? It served prior to Darwin as an expression of the dogma of special creation. Does it serve equally well now to represent concrete realities as seen from the evolutionary standpoint? Morphology is essentially a comparative science dealing with phylogenies, and the limiting of this paper to the twenty minutes allowed will permit only of a *résumé* rather than of the presentation of the detailed data as to the true significance of the species concept as related to phylogeny and evolution. On the matter of species it is to be noted that Darwinian evolution dealt rather with the question of origins than with that of content. In this particular the change is not *per se* so basic as we sometimes think. Linnaeus' idea of a species as a recognizably distinct group of interbreeding individuals all having a common descent is still the basis of most of our clear thinking in matters of classification. The evolutionary dictum that species originate as variants from a parent group and are themselves the potential parents of groups yet to be produced does not necessarily affect the question as to their make-up or delimitations at any given time.

Does the concept *species* represent as adequately the unit of evolutionary progress as it did the unit for theories of special creation? The value of a scientific concept is not infrequently in direct proportion to its plasticity as shown in its ability to undergo more or less fundamental and far-reaching changes in its significance with the increase of our knowledge of the subject data on which it is based. For example, in cytology the concept *cell* has shown its utility by surviving such fundamental modifications in content as are involved in the change from its original use as relating to a cavity or box-like chamber in plant tissue to its present use to designate a one- or several-nucleated mass of protoplasm. The changes now going on in the conceptions back of the term *atom* are another illustration in point.

I suppose there never was a time before in the history of science when a theory played such a dominant rôle as has the theory of evolution since the time of Darwin. Never has a viewpoint proved so fruitful of new interpretations, so stimulative of productive research. Its basic concepts dominate alike morphology, physiology, sociology, and such less sharply defined realms as psychology and philosophy. The only comparable case

[1] Read in the symposium on "The Utility of the Species Concept," at the joint meeting of Section G of the American Association for the Advancement of Science, the American Phytopathological Society, and the Botanical Society of America, at Toronto, December 28, 1921.

is that of the atomic theory in the physical sciences, and here it is interesting to note that the modern alchemists with their isotopes and electronic dissociations are threatening to make chemistry and physics evolutionary sciences; at least, they are striving to lay the foundations for a true evolutionary theory of the elements. All schemes of classification aim to be phylogenetic. Their rubrics are based without question on evolutionary concepts and express the knowledge of our time as to genetic relationships between the units classified.

There is no real question that the species concept as used by Linnaeus, so far as it relates to content at any one time, does fit in with the facts of evolutionary phylogeny as we know them. Modern research has made us more certain than ever that the Linnaean species do by and large constitute recognizable groups of more or less freely interbreeding individuals whose interwoven pedigrees constitute a specific fabric less diversified than that of the genus, family, or order. Only extremists deny the possibility of segregating and recognizing such genetic units. Only those who hold that all organisms constitute a web of life or vital fabric of descent in which each individual pedigree is inextricably woven up with other pedigrees so that its identity is lost in that of the whole, or, on the other hand, those who hold that the individual pedigrees are the only recognizable and differentiable units and that these are all of essentially the same order of magnitude, have really cut loose from the use of the species concept.

The question is today not whether Linnaean species are in general good phylogenetic units, but whether, with the discovery and recognition of such divisions of these Linnaean species as our small species, or subspecies, which also constitute recognizably differentiated groups of interbreeding individuals, we should now transfer the term *species* to these phylogenetic units of a lesser order of magnitude, leaving the Linnaean units unrecognized, or perhaps—as has sometimes been hinted by the "splitters" might be desirable—giving them in many cases generic rank. It is of interest to note that such proposals do no violence to evolutionary concepts. Agreement here is practically universal among all ranks of biological students. The question turns largely on what is practical. On this point Hall has put the evidence very clearly. It is indispensable in our systems of classification that we recognize and delimit units of both these orders of magnitude. The geneticists and the plant geographers can give clear expression to their results only by recognizing these lesser units—twigs on the evolutionary tree. It is in them that the variants of genetic modification and many times of geographic distribution are displayed. They, or still lesser races, varieties, etc., are the real material of evolutionary advance. Their recognition in the field and their production in the breeder's experimental plots constitute the two great current means of advance in evolutionary science. On the other hand, the recognition and critical delimitation of the Linnaean units is no less important for the geneticist and plant geogra-

pher in fixing the trends of evolutionary science and of plant and animal migration, as well as in the more popular fields of floristics and elementary botanical instruction.

There is little doubt that, if the categories represented by the Linnaean species were to be dropped and the term *species* applied exclusively to the smaller units, we should find ourselves resorting to the use of such rubrics as *species-groups*, *species-complexes*, etc.

We are passing, in my opinion, at present from a stage of knowledge in which the difficulty seemed to be to discover how specific delimitations could ever be broken down to a stage of knowledge in which our difficulty seems rather to be to find adequate evidence of delimitations between the hitherto recognized specific groups. Intensive breeding experiments and intensive study of geographic distribution have both contributed to this result. Far be it from me to enter the lists for either Mendelism or mutation in the great Oenothera controversy. But we must all agree that the mass of mutants or segregants, one or both, which have been so painstakingly described and pedigreed are well calculated to make the lay botanist, be he morphologist or physiologist, more cautious about affixing a species name to any chance evening primrose he happens to find by the wayside or in a garden. The recognition of the so-called species of Oenothera has become a matter of highly expert judgment and discrimination. In this we have certainly traveled far from the viewpoint of the immediately post-Darwinian biologists, who, while they were convinced that species come about by evolution, were quite willing to despair of ever being able to see evidence of the process going on, much less to initiate and control its course and to begin to trace out the mechanism of variation. We are confronted with a mass of evidence as to species in the making among wild plants, and we no longer hesitate to recognize that the breeder can produce, and has from the beginning of agricultural science produced, modifications of type quite comparable to those which characterize evolutionary species.

It goes without saying, of course, that from this evolutionary standpoint the physiological or biological species which are so common and so sharply marked among the parasitic fungi, and the bacterial races differentiated on the basis of their specific pathogenicities or cultural characters, are just as much to be recognized as evolutionary units as are groups differentiated by structural characters. The description and characterization of such groups is not easy, but their existence as biologic entities can not be questioned. The importance from a practical standpoint of their diagnostic characters makes sure that their careful and adequate classification will always be an attractive field of biological research.

The consideration of such groups as these leads naturally to the question as to the practicability in general of such an evolutionary system of classification as I have been arguing for. Will it ever be possible, in any considerable number of cases at least, to trace out the devious paths by

which the groups of plants and animals as we find them today have descended from those of prehistoric or geologic times? Will it ever be possible even to agree upon the delimitations of the groups as they exist today? Will not generic delimitations always be, as is commonly said, matters of opinion? Even if this be the whole story, be it noted that in the long run we may hope to discriminate between the opinions of the biological genius and those of the biological dullard.

The currency of this statement about generic limitations indicates not only the difficulty of such discriminations, but from one angle their evolutionary unimportance. So far as we know at present, it is a relatively unimportant matter how large or how much subdivided a branch of the evolutionary tree is taken for the genus unit, providing only you do not put with its twigs those from other branches. But, it may be asked, will it ever be possible to get the geneticists to agree on how many these are and how to delimit the mutants or segregants of the Oenothera species? We may best leave this specific question to the future, but that there is coming to be a fair degree of agreement as to the identity and delimitation of many Linnaean species will hardly be questioned. We are demonstrating in some cases that certain groups long suspected of free interspecific hybridization are really guilty. The difficulties of the systematists with such groups are more than compensated by the joys of the geneticists and cytologists with each such new discovery. In fact, the cytologists are even now coming to the rescue in the case of the roses, the Jamestown weeds, and the cereal grains, not to mention minor cases, with a brand new set of earmarks for the identification, characterization, and classification of the systematically difficult members of these groups. But discussion of the proper treatment in an evolutionary system of classification of hybrids and their progeny would certainly carry me beyond the limits of my allotted twenty minutes. In my opinion the systematists have been too much in haste to tie up their specific names with so-called historic type specimens. It is not impossible that we may be able to recognize and characterize in its description the actual biologic type form for a species. As you may be aware, my studies on the morphogenesis of such simple forms as the Pediastrums have led me to believe that specific types are very definite and concrete entities capable of being recognized and adequately described both quantitatively and qualitatively. This attempt to recognize and describe what is biologically typical is certainly the aim with present-day systematists who know their material in the field as well as in the herbarium, and there is little reason to doubt that characterizations so based do in general give a picture of the biologic type of the species in its relations to its subspecies, varieties, etc., and to the other related species of the genus.

It seems to me safe to assume that the work of classification will never be finished till the units large and small are brought as nearly as available evidence permits into their evolutionary sequence.

Morphology is commonly said now to be a dead science. Its last gasps have consisted of such tenuous and ill-founded speculations as to have led to general lack of interest and distrust in its conclusions. That there is too much truth in these utterances is to be admitted. Many morphologists have attempted to bridge the gaps in their knowledge with "all too bold guesses and ill-judged hypotheses."

On the other hand, it is hardly to be imagined that biologists will ever lose interest in the great problems of the evolution of plant and animal types. It is equally clear that we shall never rest content until our classifications of plant and animal species present in the fullest degree possible a picture of their evolutionary descent.

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THE SPECIES CONCEPT FROM THE POINT OF VIEW OF A PHYSIOLOGIST AND BACTERIOLOGIST[1]

GUILFORD REED

The demands of bacteriology for the classification of a group of organisms which on account of their minuteness do not lend themselves to conventional structural differentiation has occasioned the development and utilization of different criteria of organic relationship. These criteria, though they have found little favor outside this restricted field, apply equally to the classification of all organisms; and, moreover, from a physiological point of view constitute a more fundamental species concept than one based on structural differentiation. The fundamental basis of these criteria is the conception that organisms differ in the chemical constitution of their protoplasm. The methods of making the determination may be considered indirect, from chemical standards, but they are none the less precise: namely, the methods of immunology.

I

It has long been known that, when an animal receives an injection of certain substances, antigenic substances, antibodies are developed in reaction. These antibodies combine in the body of the immunized animal or *in vitro* with their antigens to produce the various familiar antitoxic, lytic, agglutinating, precipitating, and many other reactions. From the present point of view the significant feature of these reactions is their high degree of specificity: an antibody reacts only with its particular antigen and with no other. The specificity, moreover, is dependent upon the chemical identity of the antigen. Wells and others have shown that when a known chemically pure protein is used as an antigen its antibody reacts only with that chemically pure protein and with no other protein. Such reactions are now finding favor in biological chemistry as a means of identifying proteins which can not be distinguished by the ordinary analytical procedure. We have, then, in these reactions the most delicate known method of detecting chemical differentiation in the complex constituents of protoplasm.

Application of these methods to the differentiation of bacteria now constitutes an extensive and familiar literature. Any detailed consideration is out of place; a single example will be sufficient for the present thesis. If we have a sample of *Bacillus typhosus* immune serum, its agglutinins will react in high dilution with the organisms used for the immunization,

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i.e., *B. typhosus.* The maximum dilution in which it will act depends upon the degree of immunization. The serum, moreover, will not react in these high dilutions even with closely related organisms (as the other members of the typhoid-*coli* group), and will not react at all with organisms outside of this group. If in a particular sample the serum reacts with *B. typhosus* organisms in a dilution of 1-5000, it will react with the closely related *B. paratyphosus* in a dilution of about 1-200, and with *B. coli* in one of about 1-20. If typhoid organisms are added to such a serum in excess and later removed by centrifuging, both the specific typhoid agglutinins and the group agglutinins will be removed with the organisms. Similar treatment with the other bacteria of the related group will serve to remove their small content of agglutinins but will not have any appreciable effect upon the specific agglutinin.

Such results as this are confirmed by other immune reactions, and pathogenic bacteriology is a series of such examples. The recent application of this method to the study of the Pneumococci is the basis of one of the most illuminating chapters in modern medicine.

The significance of these results for the present discussion rests upon the fundamental chemical nature of the immune reaction, as previously noted. On this basis it must be concluded that the typhoid organisms react because of chemical identity with the antigenic substances of the individuals used in the immunization; closely related organisms react to a certain degree, because of chemical similarity, and less closely related forms fail to react because of chemical dissimilarity with the original antigen.

II

Although not utilized in practical taxonomy in other groups, we have ample evidence of the existence of similar distinctions. The extensive studies of Nuttall on the immune reactions of vertebrate blood are most significant. Nuttall found that when an immune serum containing precipitins for human blood serum was mixed with the latter in a high dilution, a definite volume of precipitate was formed; but when mixed with other sera the precipitate was quantitatively less according to the degree of relationship of the animals supplying the sera.

The work of Uhlenhuth, Wells, Osborne, Gohlke, and many others has brought forward an enormous amount of evidence to show that chemical differentiation exists between homologous proteins in both plants and animals.

Reichert and Brown have given us some more direct evidences of chemical specificity. Ten years ago they demonstrated that the haemoglobins of vertebrates could be distinguished by their crystallography. The results of these extensive studies indicate that there is a common structure of the haemoglobin molecule, whatever the source of the haemoglobin; that the crystals of a genus belong to a crystallographic group which represents a

generic type; and that the crystals of each species of a genus may be distinguished from those of another species of the genus. The various crystal forms depend, obviously, upon the chemical properties of the haemoglobins. More recent work of Reichert shows that specific and generic types of starch may be distinguished both by the microscopic structure of the grains and by the chemical reactions of the starch.

Such evidence, when it is all considered, is fragmentary compared with the detail of structural differentiation; but it is ample to warrant the conclusion that organisms differ in the chemical constitution of their protoplasm, and it remains for subsequent analysis to determine the full extent of this chemical characterization.

III

The increase in size of cells or organisms involves that formation of new material, which must be largely, if not entirely, synthesized within the developing cell. In many cases these synthetic reactions proceed with great rapidity, whereas outside the cell such reactions, if they occur, proceed at an exceedingly slow rate. The most generally accepted explanation attributes to enzyms the rapid rate of these synthetic reactions. Moreover, the same enzyms which operate in hydrolytic reactions are evidently equally capable of catalyzing the opposite or synthetic reaction. On this basis, therefore, it will follow that under favorable conditions those substances which a cell is able to digest may also be synthesized by that cell.

One of the most conspicuous characteristics of enzyms is their high degree of specificity. From Pasteur's classical demonstration of the relation of *Penicillium glaucum* to the tartaric acids we now have a long and familiar list of enzyms which react only with their particular chemical compounds and fail to react with other compounds. The distribution of enzyms in living cells, moreover, exhibits many limitations. This is particularly evident among the bacteria, and its utilization constitutes one of the unique features of bacterial classification. In the *coli*-typhoid group, for example, the members exhibit a wide range of activity toward carbohydrates: from species which ferment only certain monosaccharides to organic acids, to species which ferment many monosaccharides and disaccharides to carbon dioxid and water. The division of species of bacteria which liquefy gelatine, split certain nitrogen compounds to indol, and coagulate casein, from species which do not possess these enzym activities is one of the most frequently observed of bacterial processes.

The distribution of these enzyms is, moreover, a constant factor. The observation that an enzym is produced in reaction to the chemical nature of the substratum appears to be true only to a certain degree. An enzym already present in a cell may in some cases be produced in increased amounts by reaction with the substratum, or enzym action may be suppressed by the conditions of the environment; but where an enzym is normally absent

from an organism it cannot be engendered, as much bacteriological experimentation has demonstrated.

It may be concluded, therefore, that, as a cell digests or does not digest the substances with which it becomes associated, it synthesizes the substances for which it has hydrolytic or dehydrolytic enzyms.

R. S. Lillie has recently brought forward a theoretical explanation of this synthetic action which, if it can be supported, is most attractive. The cell proteins he considers exert a type of autosynthetic action, presumably by the dehydrolytic condensation of amino-acids. The energy transformation in certain of these reactions he considers to be too great to be accounted for by enzym action, and he finds the formation of electrical circuits between different regions of the cell to account better for the observed reactions.

Considerations of such phenomena as emphasized by Rettger's work on the growth of various species of bacteria in the same mixture of amino-acids and other simpler nitrogen compounds may serve to visualize specific synthesis. From such a mixture the various species grow and multiply in their characteristic form, which necessitates that they synthesize their characteristic body proteins, and these we know from serology to be chemically distinct.

IV

If species are characterized by the presence of specific chemical substances, species continuity as well as individual development must depend upon the constancy of the specific synthetic reactions. Any deviation from the characteristic reaction in a cell resulting in atypical end products will of necessity alter the chemical specificity; which alteration may be exhibited in atypical activity, pleomorphic form, or death.

Some recent data concerning the origin and nature of pleomorphic bacteria may be interpreted as resulting from modified synthetic activity. We found that the form of the influenza bacillus could be greatly altered by the H-ion concentration of the culture medium. In a medium of sufficient acidity or alkalinity to be near the growth-limiting reaction, a large percentage of the organisms appear in very distinct pleomorphic form, frequently a hundred times larger than the typical. Moreover, these atypical forms entirely resist the agglutinating action of specific serum and therefore lack the antigenic proteins of the normal cells, a deficiency evidently resulting from altered synthesis imposed by the reaction of the environment. Such an effect might be expected from the familiar action of H-ion concentration upon the proteolytic enzyms.

The transmission of chemical specificity through a specialized germ plasm has received no attention from the present point of view. Yet we might interpret Guyer's recent familiar results concerning the inheritance of experimentally induced eye defects as a case in point. Guyer prepared an anticrystalline-lens serum by immunizing fowls with macerated rabbit

lenses. This immune serum injected into rabbits produced no direct reaction; but defects of the crystalline lens appeared in the progeny of both male and female through several generations according to a definite ratio.

The immune body for anatomical reasons may not come in contact with the crystalline lens, so that we appear to have two alternatives. Either the immune body is transmitted, enters the germ as a new factor, and appears in the developing embryo with destructive effects upon the lens; or, in the injected animal the immune body combines with the lens determiner in the germ plasm and effects its destruction or alteration. If this be true, according to the fundamental basis of immunology, the immune body combines with the determiner because of chemical identity between the substance of the determiner and that of the immune body antigen, *i.e.*, the lens. In other words, the development of lens is conditioned by the transmission of chemically similar substance in the germ.

It is most significant that, in so far as chemical specificity has been determined, it is in close agreement with relationship based upon structural features. We may be permitted to conclude, therefore, that the constitution of the protoplasm is the fundamental species characteristic, of which form and structure are manifestations. This perhaps strengthens the frequent claim that physiology regards species too lightly, yet at the same time it provides a rational basis for physiological generalization and physiological specialization. We may generalize when we have to do with nonspecific reactions of protoplasm or with agencies which influence in a similar manner the various specific constituents; we must specialize when we have to do with the specific substances of the protoplasm.

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THE SPECIES CONCEPT FROM THE POINT OF VIEW OF A PLANT PATHOLOGIST[1]

E. C. Stakman

To the plant pathologist the problem of the species concept is not only of academic interest but also of intense practical importance. While pathologists as well as other botanists love to seek truth for truth's sake, most of them are under obligation to seek the truth for the good it may do in the complex process of preventing the world from starving. The delimitation of species is prerequisite to the experimentation and research of the pathologist. It is essential that the specific purity both of the host plant and of the pathogene be known. If these fundamental facts are not assured, the pathologist is likely to find not truth but error; or, at best, the truth is in danger of being badly garbled. The accurate description of species, then, is of primary importance, but it is not an end in itself, but only the means to an end. However, being the means to an end, the accurate description of pathogenic fungi is of greater practical importance to pathologists than to any other class of botanists.

It is to be hoped that there will be no unduly severe criticism on account of what may seem to some an ultra-utilitarian viewpoint. The fact is that the pathologist must learn the effect of pathogenes on the host plant. In order to do this, he first must know thoroughly both the pathogene and the host plant. He must depend, therefore, on systematic mycology and other systematic botany for the tools of his trade. If the tools be unsuitable for his work, either he must improve them or he must make his own. Therefore it is pertinent to inquire what quality of tools have been furnished him in the past.

To the taxonomist of higher plants, and particularly to the geneticist, the pathologist is indebted for the proper attitude toward the host plants which he uses. The geneticists have given us the pure-line conception for higher plants. Pathologists now are avoiding many serious mistakes by taking the simple but fundamental precaution of using pure-line host material. For example, wheat generally has been considered to be close-pollinated. But Hayes has shown that natural crossing may occur in the field. Supposing, then, experiments are made on the biologic specialization of pathogenic fungi attacking varieties of wheat: what reliance can be placed upon the results when bulk material of wheat seed is used? Some of the plants very likely will be heterozygous for resistance. This may

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lead to the deduction of entirely erroneous conclusions regarding the pathogenicity of the parasite, especially when the experiments are being made by investigators who are a little over-zealous in detecting evidence of inconstancy in pathogenes. Serious mistakes of interpretation often are made on account of the use of supposedly pure, but actually impure, host material. It is not sufficient for the careful investigator of wheat diseases, especially when fundamental relations are being sought, to know which variety he is using, but he also must be sure that he is using a single pure line of that variety.

If it is true that the greatest caution is necessary to assure the use of pure lines of host plants, it certainly is true that still greater precautions must be taken to use "pure lines" of the pathogene, because it usually is more difficult to detect genetic differences in the lower organisms. Does the practice under the present species concept give the pathologist the necessary assurance regarding the specific purity of the pathogene?

It will be agreed, at least by most pathologists, and I believe by many mycologists, that pathologists often have derived but little aid and comfort from published descriptions of pathogenic fungi. Possibly this has been due not so much to the lack of a proper species concept as to the failure properly to apply that concept. Until recently species have been delimited primarily on the basis of morphology. But the pathologist can not be content to know what fungi look like; he must know also what they can do, because that is his primary concern. He is compelled to study fungous behaviorism, and, in so far as morphological descriptions aid him in this study, they are extremely valuable. It probably is superfluous, however, to state that morphology alone no longer can be considered a sufficiently accurate basis for determining the specific purity of many organisms.

It would scarcely be profitable, even if it were possible, to define species. In general, however, as applied to fungi, the concept seems to be based on the general underlying ideas, (1) that all the individuals comprising a species are sufficiently alike morphologically to make it possible to differentiate them from individuals of other species by means of morphological characters, and (2) that the characters are relatively stable through successive generations—"a perennial succession of like individuals," according to Farlow.

The grouping of individuals into species, then, is an attempt to make it possible quickly to recognize closely related plants and to call them by name readily. The morphology of the fungus has been used as a criterion of essential similarity of individuals. And unfortunately it often also has been misused. The careless, premature, and rather reckless naming of new species of fungi has been a tremendous handicap to the pathologist. Everyone is so familiar with the unfortunate consequences of this tendency, now happily disappearing, that it is scarcely necessary to cite specific examples. Too often temporary modifications, which are not inherited at all, have

been used as a basis for the multiplication of one true morphologic species into several imaginary ones. And, on the other hand, several distinct species sometimes have been described as a single species, either on account of faulty technique or on account of the fact that the range of conditions under which the fungi were studied was not sufficiently wide to make possible the detection of differences which become apparent only under certain environmental influences. The range of variability of many species of fungi is very wide. The character of growth, the ability to reproduce, and the morphology of the organism may be influenced profoundly by the amount and kind of food available and by environmental conditions. Klebs, Thom, Coons, Duggar and his associates, and other investigators have demonstrated this conclusively.

The pathologist is vitally interested in knowing the morphology of a pathogenic organism, not only on one host and under one set of physico-chemical conditions, but under all possible conditions. And he is especially concerned with the question as to whether essential morphologic identity means also essential physiologic identity. In fact he knows that in many species it does not.

Every one now knows that there may be physiologic races within a morphologic species, and there has been a growing tendency, therefore, to use physiologic characters for the delimitation of species. The bacteria in general are separated into species on the basis of their physiological reactions, and very little objection is raised. There also is a tendency to use physiologic characters more and more in systematic work on fungi. The taxonomic work of Appel and Wollenweber, and of Sherbakoff on Fusarium, and of Thom on Penicillium was based not only on morphologic, but also on cultural or physiologic characters. More and more the description of species is being based on material grown on standard media or on several hosts, and under known conditions. Unless this is done, descriptions often mean nothing, because the so-called species may contain not only several morphologic, but also several physiologic, races.

There seem to be different degrees of specialization into biologic forms, specialized races, chemical species, *Gewohnheitsrassen*, physiologic races, or whatever one chooses to call them. These terms were not all used exactly synonymously originally, but, since the differences represented by the terms seem to be in degree rather than in kind, they are all called biologic forms in this paper. These forms supposedly are practically indistinguishable morphologically, although slight differences are known to occur; but they differ decidedly from each other in their physiologic action. There would seem to be several classes of such forms, although the categories into which they can be placed may represent no really fundamental differences.

Dox has shown that there may be such distinct chemical differences between species of Penicillium and Aspergillus that the species can be recognized more easily by chemical than by morphologic characteristics.

Among pathogenic fungi similar chemical differences apparently exist; there appear to be distinct forms of *Sclerotinia* sp., causing brown rot of stone fruits in the United States, which consistently produce strikingly different types of growth on various synthetic media. The differences between biologic forms of Erysiphe, of *Puccinia graminis*, of *Puccinia coronata*, and other pathogenic fungi, no doubt also are chemical, although the exact nature of these differences has not yet been ascertained. It is significant, however, that the reaction of several biologic forms of *P. graminis* "*tritici*" to hydrogen-ion concentration differs perceptibly. Whether the physiologic differences between biologic forms can be detected on artificial culture media or only by the action of the forms on host plants, it would seem that the nature of the difference is essentially the same—that is, physico-chemical.

Why were biologic forms not called species when first discovered? Probably because the morphological concept of species had become so firmly fixed that it was considered too heterodox to use physiologic differences as a sole basis for classification. Furthermore, morphologic differences were considered more permanent than physiologic differences. But, even as early as 1898, Farlow read the following to the Botanical Section of the American Association:

> When therefore the botanist denies that physiological species are properly species, he is practically admitting that his own definition, the perennial succession of like individuals, is used by him in a special sense, and he does not seem to be aware that species as he limits them are artificial and not natural. The belief that species should be based on morphological rather than physiological characters rests on the assumption that the former are more likely to be inherited and thus show the temporary attempts of the organism to adapt itself to the environment. It is perhaps a question whether the grounds for this belief are as valid as has been supposed. We readily see the morphological characters which have been inherited, but it is usually only by accident or experiment that we recognize the physiological or pathological qualities.

Biologic forms long were considered to be unstable. Ward, Salmon, Freeman, Freeman and Johnson, Pole-Evans, and Johnson all obtained evidence which led them to conclude that the parasitic capabilities of biologic forms of *Puccinia dispersa*, *Erysiphe graminis*, *Puccinia graminis*, and *Puccinia phleipratensis* easily could be changed by bridging hosts and by other influences. The results of these investigations indicated that biologic forms readily acquire the ability to parasitize normally immune hosts, provided they are grown first on some closely, or sometimes even distantly related, susceptible species of host plant. Thus *P. graminis tritici* is incapable of attacking oats, but can grow on barley. On barley, according to Freeman and Johnson, the rust acquires the ability to attack oats slightly, presumably on account of some chemical change in the biologic form. If biologic forms could be changed so easily, the objection to using their physiologic characters in classification certainly would be valid. But do they change easily?

The so-called plasticity of biologic forms of *Puccinia graminis* has been

investigated thoroughly. For ten years the writer and various colleagues have tried in every conceivable way to change the hereditary parasitic capabilities of *P. graminis tritici* and *P. graminis secalis*. Considerable work also was done with *P. graminis phleipratensis*, *P. graminis avenae*, *P. graminis agrostis*, and *P. graminis tritici-compacti*. It was impossible to induce hereditary changes, or, indeed, any fundamental changes, although the growth of these fungi, like that of other plants, is influenced by environmental conditions. These biologic forms were as constant genetically as were the species of wild and cereal grasses upon which they were cultured. There was no evidence whatever that the inheritance of physiologic characters by these biologic forms depends any less upon real germinal specificity than does the inheritance of structural characters in morphologic species. Reed states that "in studying the races of *Erysiphe graminis* one also gets a strong impression of their constancy and definiteness and they seem as real as though separable by structural features." Dox concluded that species of Penicillium and Aspergillus could not acquire new ability to produce enzyms by any special methods of nutrition; and Brierley was unable to "educate" *Botrytis cinerea* unless the initial culture consisted of a mixed population, although a form with colorless sclerotia did suddenly appear from a single-spore strain. This phenomenon, however, can be explained on the basis of known principles of genetics. Brierley points out clearly that it is quite essential to use pure lines of the organism in "fungus-educability" studies. This point can not be emphasized too strongly. Any one is likely to obtain very striking evidence of rapid changes of biologic forms unless his supposed biologic form itself is pure. For example, until a few years ago it was supposed that the *tritici* form of *P. graminis* could change readily. But the so-called *P. graminis tritici* itself consists of at least thirty-seven biologic forms which can be distinguished from each other readily by their action on certain pure-line varieties of various species of *Triticum*. All of these forms develop normally on various pure lines of *Triticum compactum* and apparently also on several wild grasses. It would be strange, in using such mixed cultures, if changes were not observed. Those hosts which were attacked by several of these forms naturally would appear to act as bridges to the normally immune forms. The longer one works with these forms, the deeper becomes the conviction that they represent as real, as constant, and as genetically pure entities as do morphological species.

But many biologic forms differ from each other not only physiologically but morphologically as well. The forms of *P. graminis* which are separable on the basis of their action on different genera of host plants (in the United States) can be recognized by the size, shape, and color of the urediniospores and also by the size of teliospores and aeciospores, provided these spores are developed on hosts of the same approximate degree of susceptibility and in approximately identical environmental conditions. The differences

between some forms may be only two or three microns, but these differences easily can be recognized by quantitative methods; and they are as constant as are the differences between many recognized species of fungi. There is a consistent average difference of ten microns between the length of the urediniospores of the *tritici* form and that of urediniospores of the *agrostis* form. But even if there were no morphologic differences, these biologic forms are distinct and constant pathogenically, and we must recognize their existence.

In a recent paper Brierley expresses views similar to those expressed in this paper and makes the concrete suggestion that Lotsy's terminology, proposed for the phanerogams, be modified to meet mycological needs as follows. He suggested that the term *linneon* replace the species in the Linnaean sense—the description being based on morphological grounds only; *jordanon* would be based on morphological characters which were demonstrated to be transmissible; and *species* would be established only on the basis of morphologic and physiologic reaction under standardized conditions. The term *modification* would be used to designate non-transmissible effects of external conditions. Whether or not this terminology is adopted, the principles involved are worthy of serious consideration.

The physiological concept already has been added to a certain extent to the morphologic concept of species. We raise no particular objection to basing the determination of a species of the Uredinales partly on life history, and all pathologists use physiologic characters in establishing species of phytopathogenic bacteria. If everything which the pathologist must know is classified, it will be necessary to add more and more of the physiologic concept. The simple fact is that as scientists we ought to want to classify plants on the basis of those characters which are really characteristic, whether they be morphologic or pathologic, and as practical pathologists we must do so. We shall no doubt encounter difficulties, but, as technic becomes more standardized and refined, it will become possible to recognize still less obvious differences in species of pathogenic fungi than we now do.

If the criticism be made that the proposed recognition of physiologic characters in classification would be drawing too fine distinctions, all that can be said is that the real distinctions were drawn by Nature; and, if we are dealing with pathogenic fungi in a practical way, we must recognize these distinctions; and, if we are seeking the ultimate truth regarding fungi, surely we ought to accept it in plant behavior as well as in plant structure.

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THE RELATION OF THE ENZYM PECTINASE TO INFECTION OF SWEET POTATOES BY RHIZOPUS

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It has been demonstrated that, if the proper conditions are maintained in the storage house, sweet potatoes can be kept for a considerable length of time with practically no loss from soft rot. If, on the other hand, the conditions are unfavorable, infection followed by decay may take place.

The cause of the soft rot has long been suspected to be due principally to *Rhizopus nigricans* Ehrb. (14, 19). Before positive proof of its parasitism was obtained, some difficulty was experienced in isolating the organism from the rotting potato and still more in obtaining infection by it. If, as is customary in pathological technique, the plate plantings were made from tissue close to the healthy zone, a sterile culture was almost invariably obtained. If, on the other hand, the plantings were made from rotted tissue several millimeters back of the undecayed tissue, a pure culture of the organism was usually obtained. This suggested that there was some "action in advance" of the growing hyphae. Harter and Weimer (17) showed that this action or dissolution of the middle lamellae in advance of the growing hyphae is due to a substance of the nature of an enzym, which, following the precedents established by Bourquelot and Heressey (1), Jones (22), Euler (11), Zeller (37), and others, they have designated as pectinase.

Bruschi (6) found that the cells of plums on which *Monilia cinerea* (Bon.) Schroter had grown for two days were separated along the line of the middle lamella, and concluded from her results that the enzym pectinase was produced by the fungus. Munn (28) obtained similar results with the fungus causing the neck rot of onions. He demonstrated the production of oxalic acid, but from the nature of its action came to the conclusion that it has little or nothing to do with the maceration of the tissue. Munn clearly demonstrated that an enzym which he calls pectinase was responsible for the disintegration of the tissue noted.

Although *Rhizopus nigricans* has been quite generally accepted as the cause of the soft rot of sweet potatoes, its causal relation has been somewhat difficult to prove. The usual method of placing spores or spores and mycelia upon the unbroken surface or upon a wound usually gave negative results, even when the potatoes were placed in a moist chamber lined with wet filter paper. No better results were obtained when the spores and mycelia were injected deeply into a wound made with a needle or forceps. If, however, the method described by Harter, Weimer, and Adams (19), in which the fungus was grown for one or two days in sweet-potato decoction

and then poured into a fresh "well" made into the potato, was followed, positive results were usually obtained. These results suggested strongly that an enzym was secreted which immediately began to macerate the host tissues in advance of the growth of the fungus. That dissolution of the middle lamellae takes place in advance of the mycelium is further borne out by the fact that a sterile zone of uncertain width is always present between the healthy tissue and the tips of the hyphae. In view of the fact, first, that an enzym seems to play such an important rôle in the decay of sweet potatoes after infection once takes place, and, second, that the original infection is difficult to obtain without first growing the fungus for one or two days on a nutrient medium in which the enzym is secreted, the writers proposed to make a detailed study of the part played by the enzym pectinase in infection.

It should be pointed out here that, while the experiments dealing with this phase of the subject were made with either *R. nigricans* Ehrb. or *R. tritici* Saito, several other species also were found capable of decaying sweet potatoes (20) as well as a number of other vegetables and some fruits. All the different species were found to secrete pectinase (18) and to bring about a maceration of the host tissue in a similar way to that caused by *R. tritici* and *R. nigricans*. It is therefore believed that the rôle played by the enzym secreted by these two species is typical of that played by the other species of the genus.

Pathological Histology

Sweet potatoes decayed by Rhizopus are at first rendered very soft and stringy, water often dripping out of the potato when the skin is broken open. At the outset the color of the tissue is not changed, but later it turns a cinnamon to chocolate brown. On the escape of moisture the cells collapse, the potato dries up, and the whole mass finally becomes hard and mummified. Observed in this stage, it is often classed as dry rot.

That the tissue is killed in advance of the fungous threads has been demonstrated by different methods. Attention has been called to the fact that plate plantings made from decayed tissue adjacent to healthy tissue were usually sterile. "Action in advance" was likewise demonstrated by a microscopic examination of stained sections made through the healthy and the adjacent decayed tissue in which the fungous threads were colored blue by Pianese's stain. The results showed that maceration of the tissue had taken place several cells beyond the most advanced hyphae. Furthermore, similar sections stained in methylene blue demonstrated the presence of the middle lamella connecting the cell walls of healthy tissue, while in the decayed tissue it had completely disappeared.

If bits of decayed tissue floating in water are examined microscopically, the cells, although themselves remaining intact, will be seen to be separated from each other along the plane of the middle lamella. So far as the

writers' observations go, the hyphae pass between the cells but do not penetrate, at least during the earlier stages of decay.

Investigations by the writers have demonstrated that neither *Rhizopus nigricans* nor *R. tritici* produces cytase, which may account for the fact that they do not penetrate the cell wall. The absence of a cellulose-dissolving enzym was demonstrated in several ways. Some preliminary and somewhat crude experiments were conducted in which it was attempted to grow Rhizopus on a nutrient solution with Whatman chemically prepared filter paper as the only source of carbon. A modification of Czapek's nutrient solution in which ammonium sulphate was substituted for sodium sulphate served as a medium. Rhizopus makes a rapid and profuse growth on this medium with glucose or even starch paste as a source of carbon. When the filter paper was substituted for glucose, the fungus made practically no growth. The filter paper appeared to be unaltered, and no reducing sugars could be detected according to the method of Clark (8). On the other hand, when starch paste, for example, is used as a source of carbon, reducing sugars are formed in advance of the needs of the fungus (18).

A second series of experiments were carried out in which the action of Rhizopus on cellulose was studied. A cellulose agar was prepared according to the method of McBeth and Scales (26) from a good grade of filter paper as prepared by Scales (31). A uniform distribution of the flocculent cellulose imparted a milky appearance to the agar. Kellerman (25) showed that if this kind of agar in test tubes is inoculated on the surface with *Penicillium pinophilum* Hedg., the enzym cytase is excreted which eventually clears the medium by the dissolution of the cellulose. Furthermore, he found that, if discs of the clarified agar were transferred sterile to cellulose agar in plates, the cellulose in the latter would likewise be dissolved, thus proving conclusively that cytase was produced. The writers duplicated the experiment of Kellerman with *Rhizopus nigricans* and *R. tritici* but no dissolution of the cellulose took place, which would seem to indicate that the enzym is not produced.

It was suspected that perhaps in the absence of available carbohydrates these organisms were unable to make sufficient growth to produce the enzym, and that, if they were cultivated on a medium on which they would grow independently of the cellulose, they might digest the cellulose. With this possibility in mind the cellulose was added to beef agar and the same test as before was applied, with negative results. The results of these experiments seem to prove the absence of cytase production in the species *nigricans* and *tritici*.

Mode of Infection

The results of different investigators have shown that fungi may enter the host tissue, first, by way of the stomata; second, by penetrating mechanically the unbroken epidermis; third, through both the stomata and the epidermal cells; fourth, by means of an enzym secreted by the fungus which

dissolves the epidermal cells, thereby permitting the germ tube to enter; and fifth, by means of wounds. Blackman and Welsford (2) found that the germ tubes of spores of *Botrytis cinerea* Pers. in turnip juice on the leaves of *Vicia faba* L. penetrated mechanically the unbroken epidermis. Initial infections were never found to occur through the stomata. However, after the penetration of germ tubes through the epidermis had reduced their resistance to infection, the hyphae were seen to enter through the stomata. Dey (10) and Weimer (35) showed that *Colletotrichum lindemuthianum* (Sacc. and Wagn.) Scribner and the basidiospores of *Gymnosporangium Juniperi-virginiani* Schwein. penetrated mechanically the unbroken epidermis of the bean and of the leaves of healthy apples respectively. From the results of these investigations there appears to be little doubt that certain fungi have the power to penetrate the epidermis mechanically independently of an apparent enzymic action. Other fungi have been found to gain an entrance through the stomata. Jones, Giddings, and Lutman (23) picture the entrance into the leaf of the potato by *Phytophthora infestans* (Mont.) De Bary both by way of the stomata and by the penetration of the epidermis. Harter (15) observed the germ tube of *Diaporthe phaseolorum* (C. & E.) Sacc. entering the leaf of *Phaseolus lunatus* L. only by means of the stomata, and Gardner and Kendrick (12) found that *Bacterium exitiosum* Gardner & Kendrick entered the leaf through the stomata, although entrance of the fruit was accomplished only through a wound.

That wounding plays an important rôle in the infection of many crops by various fungi is well recognized. There are in fact organisms often regarded as weak parasites which can infect only through a wound. Even those organisms which are able to penetrate the epidermis or those which usually enter through the stomata frequently gain an entrance to the host through a wound. Hurd (21) found that an unbroken seed coat of wheat or barley ordinarily affords absolute protection against attacks on living seeds by Penicillium or Rhizopus in damp storage, in the soil, or in blotter germinators. Infection of such seeds was obtained, however, by retarding germination of the seed by means of low temperatures. Orton (30) found that infection of the Irish potato by *Rhizopus nigricans* was accomplished only through some abrasion in the skin.

The part played by enzyms in infection of the host has been a subject of study and investigation for a long time, and some of the earlier investigators noted what they believed to be cases of the entrance of a fungus only after the epidermis had been softened or weakened by means of an enzym. Busgen (7) and Ward (34) believed that the germ tube of *Botrytis cinerea* accomplishes its entrance into the tissue of the host with the aid of an enzym which dissolves the epidermal walls of the host. Voges (33) and Miyoshi (27), working with Fusicladium and *Botrytis cinerea* respectively, are of the opinion that softening of the cuticle by an enzym or some

dissolving agency precedes the penetration of the cells. Miyoshi was able to show that *B. cinerea* could penetrate mechanically a membrane such as gold leaf. The work of De Bary (9), Nordhausen (29), Smith (32), and others has been referred to in this connection in other papers and will be reviewed only briefly here. De Bary noted that the expressed juice of certain plant organs which had been decayed by *Sclerotinia libertiana* Fuckel if heated was no longer active, and therefore concluded that the cell-wall-dissolving action is due to an enzym. A toxic action was also noted, but to just what this action is due he was uncertain. Nordhausen as well as Smith claimed the production of a toxic substance which penetrates the cuticle and kills the underlying cells.

Some interesting results in this connection are those of Brooks (3), who attempted to infect lettuce by placing spores of *Botrytis cinerea* from a grape-extract medium, dry and in drops of water, on the leaves of plants kept uncovered in a greenhouse. Although some of the spores germinated, no infection was obtained, even when the plants were confined under a bell jar. If, however, young mycelia were placed in drops of the grape-extract medium upon healthy leaves, infection took place. Wounding insured infection. He says:

> In such cases the juices which exude from the wounded areas provide saprophytic nourishment for the further development of the germ tube. Infection could be brought about on leaves which had only just begun to turn yellow, but not on normal green leaves. Botrytis can not be considered a true parasite since it kills tissue in advance of the growth.

The results of the investigations cited above show that some organisms can infect although there is no apparent injury of the outer layer of cells of the host, while others require a wound. That Rhizopus belongs to the latter class has been demonstrated repeatedly in the following manner. Sweet potatoes of the Yellow Jersey variety, one of the most susceptible varieties, which had been cured in the usual way in the storage house, were used in these experiments. Sound potatoes were carefully washed, and a glass ring about one centimeter deep and one and one half centimeters in diameter was sealed on an uninjured spot on the surface by means of a wax made of beeswax and vaseline. A 48-hour-old culture of *Rhizopus tritici* grown in about 2 cc. of sweet-potato decoction was poured into the glass cell, which was then covered with a cover slip held in place by vaseline. In no case did infection take place. This seems to prove that this fungus is unable either to penetrate mechanically the unwounded skin of the potato or to secrete an enzym which will dissolve it.

A mature sweet potato has no true epidermis. Instead of an epidermis, which probably sloughed off early in the growth of the potato, there is a layer of cork two to four cells deep. This layer, which will be designated hereafter as the skin, is able to withstand the attack of the fungus itself or digestion by any of its secretions. A sweet potato inoculated with *Rhizopus tritici* will entirely decay with the exception of the skin, which the hyphae

are unable to penetrate even from within. If, however, some of the outer cells are ruptured, the fungus grows out and forms sporangia on the surface. Likewise, if sections of sweet potatoes cut to include some of the skin are immersed in an extract of the fungous hyphae or in a solution on which the fungus has grown, all the tissues except the cork are macerated and separate readily from it. The skin, therefore, forms an effective barrier to the penetration of Rhizopus.

Small dead rootlets were proved to be the point of entrance of the fungus in a small percentage of the trials made. These experiments were conducted by the use of the method just described. A glass ring was sealed over an old dead rootlet, and a 48-hour-old culture of the organism grown in about 2 cc. of sweet-potato decoction was poured into the cell, which was then covered with a cover slip. Out of a large number of such trials only about 25 percent of the potatoes thus inoculated became infected, while 100 percent of the controls inoculated by the well method decayed. It may be concluded from these results that the rootlets do not form as effective a barrier to the penetration of the fungus as the unbroken skin, although it was shown by Weimer and Harter (36) that a somewhat incomplete cork layer is laid down beneath the rootlets if the proper conditions of temperature and humidity are maintained. It was also shown that if the solution on which the fungus had grown was poured into a glass cell sealed over a dead rootlet, more or less softening of the tissue took place in some cases. In other words, the dead rootlets formed a point of entrance which was not in all cases effectively protected by a cork layer. A number of sweet potatoes from the storage house, which had some wounds made during digging and storage but no apparently fresh ones, after being held under running water to remove some of the dirt, were immersed in a sweet-potato decoction on which the fungus had grown for 48 hours. After about 24 hours in this solution, there was softening at the end where the potato was separated from the stem, in bruises and wounds made during digging and handling, and at certain places where small rootlets had died. These results show that in practically every potato certain wounds are present through which the enzym can enter; *i.e.*, the skin which forms the only barrier to the entrance of the fungus is ruptured.

A study was made of the extent of wounding necessary to permit infection when the fungus was grown for one or two days on sweet-potato decoction and the decoction and mycelium were used as an inoculum. Different types of wounds were tried. When such a growth was poured into a "well" made by means of a cork-borer, infection usually resulted. On the other hand, only about 50 percent of the attempts to inoculate sweet potatoes through a small scratch just sufficient to rupture the skin were successful. When the skin was punctured once with a needle, the percentage of infection was even less (35 percent). These results show that a very small wound is sufficient to permit infection if the enzym is present. No infection

resulted when similar experiments were conducted using spores and hyphae in water and in the decoction instead of the one- and two-day-old cultures. Weimer and Harter (36) showed that cork is formed over wounds when the proper conditions of temperature and humidity are maintained. This wound cork was likewise found to exercise some resistance to infection by Rhizopus, but not complete protection against invasion. Numerous observations of sweet potatoes in storage houses led to the general conclusion that infection there takes place at the ends of the potato more frequently than elsewhere, which would seem to indicate that what wound cork is formed there, together with the latex congealed over the surface, is not a complete protection against infection. Experiments in which 24-hour-old cultures of the fungus grown on sweet-potato decoction were poured into glass cells sealed over the ends of potatoes which had been in storage for some weeks showed that infection could take place through the ends.

The results thus far show that infection takes place only through wounds. Furthermore, it is evident from the results that the wound need not be large, a mere needle prick being sufficient if the enzym is present.

Saprophytic Start

When sweet potatoes are dug, each one is wounded where it is broken from the vine. Furthermore, the skin of the potato is likely to be ruptured to some extent in the ordinary farm operations of digging, handling, and storing. The results of inoculation experiments with *Rhizopus tritici* on sweet potatoes and other crops show that it seldom if ever infects except through a wound of some sort. However, inoculation experiments have conclusively demonstrated that a wound alone is not sufficient to insure infection. Hundreds of attempts to infect sweet potatoes by smearing spores alone, and spores and hyphae dry, on the surface of a fresh wound have for the most part been unsuccessful, even when the potatoes thus inoculated were subjected to the temperature best suited to the growth of the organism and to a relatively high humidity such as that obtained by confining them in a moist chamber with wet filter paper on the bottom.

Furthermore, if water in which spores and hyphae are suspended is poured into a "well" made into the potato by means of a cork-borer and covered with a cover slip to prevent evaporation, consistent infection does not take place. Likewise, if sweet-potato decoction is used instead of water, only a small percentage of infections results, in spite of the fact that the spores in both cases germinate and that those in the sweet-potato decoction form a considerable amount of fungous growth. Sweet potatoes absorb liquids quite readily, especially through a cut surface, and in all cases such as those just described the liquid was absorbed by the end of 24 to 48 hours. Whether or not this is the reason why infection does not take place under the conditions of these experiments is not clear. However, results which throw some light on this question were obtained by experi-

ments in which a strip of cheesecloth with one end in a beaker of water and the other in the wound over the spores kept the spores constantly wet. Germination took place readily under these conditions, but infection did not occur. Likewise, when cut potatoes were immersed in a spore suspension and then confined in a moist chamber, only a very low percentage of infection was obtained. As a matter of fact, the total percentage of decay by the use of this method was no greater than that of the controls which were not inoculated.

The results of the investigation so far show that sweet potatoes are difficult to infect by the usual laboratory methods. On the other hand, potatoes wounded during the winter and kept in a commercial storage house frequently decay. The interesting question in this connection is why it is so difficult to infect sweet potatoes inoculated by the usual laboratory methods while those freshly wounded and held in a storage house decay so readily.

Keen (24) stated in respect to the decay of sweet potatoes caused by *Rhizopus nigricans* that the organism must first have a saprophytic start in order to become a parasite. He found that, if the spores were germinated and allowed to grow for a short time in orange juice, and were then transferred to slices of sweet potatoes, decay would take place. Brooks (3) was likewise unable to infect lettuce with *Botrytis cinerea* by the use of spores alone, but if young mycelia were transferred to drops of grape extract, infection resulted. Neither of these investigators attempted to explain the principle underlying the "saprophytic start," but it would seem that both are cases in which an enzym played an important rôle.

So far as sweet potatoes are concerned, infection probably rarely takes place by the entrance of the hyphae directly into the healthy tissue, either wounded or unwounded. Many experiments, some of which have already been referred to, have shown that, even when potatoes are cut in two and dipped in a suspension of Rhizopus spores, infection, if it occurs at all, begins not on the cut surface but at some point at the edge of the cut where there is a bruise or dead tissue which serves to give the organism a saprophytic start. Such a "saprophytic start" is likewise furnished when the fungus is grown for one or two days in sweet-potato decoction. As already pointed out, when this method is followed, the decoction and fungous growth being confined in a "well" made into the potato, infection is practically assured. That this method is not the only one that enables the fungus to infect is evident from the following experiments. A number of potatoes were cut in two, and the cut surface of one half was held over a Bunsen burner until slightly charred. Treatment of this sort killed the tissue for several cells beneath the surface. All the halves of the sound and burned surfaces were smeared with spores of *Rhizopus tritici*. In 20 hours the fungus was growing on the surfaces of the burned potatoes, and at the end of 2 days these potatoes were about three fourths decayed. The control halves, whose surfaces were not charred, remained sound.

Rhizopus grows readily on almost any kind of culture medium. A synthetic medium made somewhat acid to prevent the growth of bacteria is generally used in isolating the different species from dead or decayed material. Sweet potatoes which have been carefully washed have been dipped into a suspension of Rhizopus spores in synthetic agar. Infection and decay of potatoes so treated have always resulted. In these experiments no fresh wounds were made, infection in every case taking place through old wounds or at points where there were dead rootlets. A sweet potato completely covered with a coating of agar is under an abnormal condition, since respiration is largely cut off. Oxygen starvation is in the end inevitable, and the resistance of the host is doubtless greatly reduced. Other experiments were conducted in which agar with spores suspended in it was placed on a fresh wound. Decay took place as in the former case but not in so short a time, since infection occurred at only a single point. These and other experiments which will not be detailed show that if Rhizopus is given a saprophytic start, either by growing one or two days in a decoction in agar on a wounded surface of the host, or in dead cells on the host, infection will almost invariably take place.

The Relation of Certain Other Factors to Infection

It is obvious that a temperature that will permit of the growth of the fungus must be maintained. The optimum temperature varies with the different species, as shown by Harter, Weimer, and Lauritzen (20). It is generally assumed that relatively high humidity is required for infection, but there is some evidence to show that infection will take place when the humidity of the air is comparatively low. Also, infection frequently fails when the humidity is relatively high. In fact, it is well known that sweet potatoes will become infected in the storage house under what might be regarded as fairly dry conditions. On the other hand, sweet potatoes, after being immersed in a spore suspension, often fail to decay when confined in a moist chamber lined with wet filter paper. Differences in the method of treatment previous to immersion in the spore suspension have given quite opposite results. If, for instance, sweet potatoes are cut in halves with a knife, dipped in a spore suspension, and then confined in a moist chamber, infection usually does not take place. If, on the other hand, the potatoes are struck against a blunt edge so as to make a wound ¼ to ½ inch deep, and are then dipped in a spore suspension, infection almost invariably results. In the former case, the surface of the potato probably dries off by the absorption of the water before the spores can germinate and infect. In the latter case, on the other hand, the mutilated cells and the cell sap form a substratum which retains sufficient moisture to permit the germination of the spores and to provide for the subsequent growth of the mycelium.

Reference has already been made to some experiments in which the

potatoes, after being cut in halves and dipped in a spore suspension, were confined in deep preserving jars. Some of the jars were plugged with cotton, some were left open, and others were closed with a close-fitting glass stopper. No decay took place, either in the jars that were left open or in those that were closed by glass stoppers. In the former case, some hyphae grew on some of the potatoes but no infection resulted. In the latter case, no hyphae were seen, which was probably due to the fact that the carbon dioxid which accumulated in the jar was injurious to the fungus. Saturated filter paper or cotton in the bottom of the jars did not materially increase the percentage of decay. If, however, air is constantly pulled through water and then through the jar, infection takes place. On the other hand, air circulation was unnecessary when the germinated spores and decoction on which they grew were poured into the "well," since it was found that, if the cover glass over the well was sealed on airtight, infection would still take place.

The Part Played by Enzyms

In this connection the writers have in mind pectinase, which they have shown is abundantly produced by Rhizopus, and which they have found is capable of macerating the tissue of sweet potatoes (18). It has been demonstrated that a part of this enzym is exuded from the mycelium into the substrate; also that a watery extract of the mycelium and the enzym exuded into the solution on which the fungus had grown would disintegrate the tissue of thin sweet-potato disks in from 2 to 4 hours. It is likely that pectinase passes into the substrate almost immediately upon the germination of the spores, and it is not unlikely that it may diffuse from the spores even before germination, since it has been shown that a watery extract of the spores will macerate raw sweet-potato tissue[1] in the same identical way as an extract of the hyphae or as the solution on which the fungus has grown. Although the exudation of pectinase into the substrate from living ungerminated spores has not been demonstrated, it has been shown that it is exuded into the solution soon after germination. The following experiment was designed especially to throw some light upon this question. A large volume of spores of *R. tritici* was suspended in sweet-potato decoction in 2-liter Erlenmeyer flasks and incubated at 35° C. After 6 hours, the decoction from one of the flasks was filtered through no. 2 Whatman filter paper to remove the spores and mycelium. Raw sweet-potato disks ½ mm. thick and 1.5 cm. in diameter were partially macerated in the solution in 24 hours. A control of the same solution steamed to inactivate the enzym produced no maceration. Only a part of the spores were germinated, with germ tubes varying from one to several times the diameter of the spore. At this stage it is to be expected that there would be only a minimum amount of pectinase present. The strength of the macerating

[1] Results not yet prepared for publication.

principle would naturally depend upon the number of spores per unit volume of solution.

The second flask was taken off at the end of 7 hours, the solution was filtered, and raw sweet-potato disks from the same source and of the same size were suspended in it. Maceration of the tissue in this case was much more rapid, being quite advanced in 24 hours and complete in 45 hours. The spores were mostly germinated, and the amount of hyphal growth was far more abundant than in the flask removed an hour earlier. A duplicate experiment with *R. nigricans* gave similar results, although the rate of maceration was somewhat slower than in the case of *R. tritici*. It would seem from these results that a substance capable of dissolving the middle lamellae is secreted early in the process of germination, and that this macerating principle very rapidly increases in amount at least in the early period of growth. The maximum is reached in about 2 days in the solution and in about 3 days in the mycelium (17).

It has been shown that this enzym is produced early in the germination of the spores (6 hours). More recent researches (not published) have shown that the spores themselves independently of their germination contain an enzym which, when extracted with water, will bring about the disintegration of raw sweet-potato disks.

The data presented thus far show fairly conclusively that Rhizopus is unable to penetrate mechanically the unbroken skin of the sweet potato. It also shows that, even though the spores and hyphae alone are placed on a fresh wound, infection usually does not take place, although what might be regarded as favorable temperature and humidity are provided. It was, however, shown by a number of experiments that if the fungus is grown for a day or two on a decoction made of sweet potatoes, and this, together with the mycelial growth, is poured into a "well" in the potato, infection will almost always take place. The writers showed elsewhere (17) that if raw sweet-potato blocks were immersed in the substratum on which the fungus had grown, after it had been freed of the fungus, or in a watery extract of the dead mycelium, a rapid dissolution of the middle lamellae took place. This action on the living tissue is in every respect identical with that produced when the fungus itself is decaying the potato. Attention was also called to the fact that in decaying sweet potatoes there is a zone of disintegrated tissue adjacent to the sound tissue which is sterile of the fungus. These and other results show that for this fungus at least a saprophytic start is nearly if not always required before infection takes place. That enzyms play a part in the decay of various plant organs is pretty generally agreed, and that they play an important rôle in infection has been suggested. De Bary, Ward, Nordhausen, Smith, and others noted what undoubtedly was enzymic action. Although the evidence as presented by them does not unqualifiedly prove the action of an enzym, there can be little doubt that they were dealing with what the writers have regarded

as an enzym. Brown (4) has shown that the germ tube of *Botrytis cinerea* is unable to affect chemically the cuticle of the host, nor does it secrete any toxic substance which can pass through the cuticle and bring about the death of the underlying cells. He found that the fungus is unable to affect the underlying tissue until the obstacle offered by the cuticle has been removed. Penetration of the cuticle must take place in a purely mechanical way. In this connection, in another paper (5), he says:

> Once penetration of the cuticle has taken place the problem becomes simply an enzymological one. Further, in the case of the so-called wound parasites the problem presented is much simpler as the problems which arise antecedent to the penetration of the cuticle do not come into consideration.

Gortner (13), in similar studies, found that *Sclerotinia cinerea* when grown on prune- and apple-juice media elaborated a very active pectase. When the fungus penetrates the host tissue it dissolves the middle lamellae, forming a product which, instead of being assimilated as food, is precipitated as a certain compound of calcium pectate. Rhizopus is one of those organisms that are unable to make their way into plant tissue which has not previously been disintegrated by enzymic action. In view of this fact, the fungus evidently depends upon the secretion of the enzym antecedent to infection. When exposed to sufficient moisture and to the proper temperature, the spores germinate. Although the spores may germinate on a fresh wound, they seldom make sufficient growth to infect. The results seem to indicate that infection must start where there are dead cells or tissue on which the fungus can grow. In these dead cells the growth of the hyphae is accompanied by the secretion of the middle-lamella-dissolving enzym. This enzym secreted by the growing mycelium, when once it comes in contact with the healthy host cells beneath, brings about a disintegration of the tissue which is later invaded by the hyphae.

Summary

1. Rhizopus can not infect sweet potatoes through the unbroken skin. Spores and hyphae smeared on a freshly cut surface will produce infection only rarely. However, when the fungus is given a saprophytic start by growing on dead rootlets, in synthetic agar solidified on the cut surface of the potato, or in dead cells killed by charring over a Bunsen burner, infection takes place readily. Furthermore, infection can be brought about readily by growing the organism for one or two days in sweet-potato decoction, if the decoction and mycelium are poured into a "well" made in the potato and then sealed over with a cover glass to prevent evaporation. Infection is accomplished only after the dissolution of the middle lamellae by means of an enzym (pectinase) secreted by the growing hyphae. In practically all cases infection takes place in wounds where there is some dead tissue upon which the fungus can get a saprophytic start. During the growth of the mycelium in these dead cells, the enzym is produced which, when it

comes in contact with the living cells of the host, dissolves the middle lamellae; the cells then die, and a suitable substance for the further development of the fungus is provided.

2. The practical significance of these results is that wounding is a preliminary necessity to infection. Although sweet potatoes are necessarily wounded at digging time when they are broken from the stem, other wounds made by rough handling during harvesting, storing, and preparing for the market should be avoided as much as possible.

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THE INHERITANCE OF FLOWER TYPES AND FERTILITY IN THE STRAWBERRY[1]

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In a study of sterility in the strawberry begun by the writer in 1914, a portion of the work was directed toward determining the underlying factors causing "nubbins" or imperfectly developed berries. These are commonly produced from the tertiary and later flowers of the inflorescence of many cultivated varieties of strawberry and result in considerable loss of fruit toward the close of the picking season.

A study of the fruiting habit of the wild American strawberries and of the cultivated varieties proves conclusively that the production of nubbins is directly related to pistil sterility, and that pistil sterility is decidedly more prevalent on plants with certain flower types than on others (7). Therefore, since the question of fruitfulness in the strawberry is primarily one of sex, a thorough knowledge of the flower types and of their inheritance is essential to the strawberry breeder if his work is to be other than blind crossing and selecting for chance high-yielding clones.

The work on the inheritance of flower types in the strawberry has been discontinued by the writer, but, as some facts have been determined, he presents the data obtained and the conclusions drawn from them.

Flower Types in the Strawberry

In the cultivated strawberry, pistillate and perfect flowers are commonly encountered. The pistillate flowers bear small abortive stamens which have never been observed by the writer to produce pollen. The pistils are generally very fertile, producing perfect fruits from most of the flowers and comparatively few nubbins. The perfect-flowered varieties develop anthers which produce varying amounts of normal pollen. As a class, these varieties are less fertile than the pistillate varieties and produce a higher percentage of nubbins and of sterile or male flowers (7).

The wild species of American strawberries may be divided into two types: those which bear only perfect flowers, as *Fragaria americana*, and those which are dioecious. The pistils of the former species are very fertile, and nubbins or sterile flowers are seldom seen. The dioecious types produce pistillate plants and plants which apparently are hermaphrodites but are in fact staminates. The pistils of the pistillate clones are usually fertile, but the pistils of the staminate clones are rarely so, and the few berries

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developed are practically always irregular nubbins. These clones are somatic hermaphrodites; *i.e.*, they appear to be perfect but the pistils are sterile. Occasionally other flower types are found in *F. virginiana*, such as completely sterile clones in which pollen formation never proceeds farther than the tetrad, but such forms are comparatively rare and need not be considered (7).

Material

The material to be considered in this paper was the result of crosses made at the Minnesota Agricultural Experiment Station in 1915 and 1916. The seedlings were later transferred to the Minnesota State Fruit-Breeding farm at Zumbra Heights. The individual plants were set in the field where they were allowed to multiply for one season. The following season, records were made as to the sex condition and the degree of fertility of the various clones. In nearly all cases the records obtained are the result of observations of the sex condition and fertility of several plants of each clone, and therefore probably represent fairly accurately the sex condition of these clones under field conditions. A few which did not blossom in 1917 were left until 1918 when final records were taken. The writer is indebted to Mr. John Bushnell for completing the records on certain crosses in 1918 while the former was absent in military service.

Terminology

For the sake of convenience and clearness in discussing the crosses of the various flower types, symbols will be used to represent the various sex determiners. Shull (4) suggested in the case of Lychnis the use of the symbol *FF* to represent a female and *FM* to represent a male, as he determined that the females were homozygous for the sex determiners whereas the males were heterozygous. Similar symbols have been found appropriate in interpreting the sex condition in the grape (5). It will be apparent from the following discussion that these symbols will not apply in the case of the strawberry, since the females are apparently heterozygous for the female and male determiners and the males homozygous. Therefore the symbols *FM* and *MM* will be used to designate the genetic condition of the female and male plants respectively of the dioecious wild forms, and *FH* and *HH* the females and hermaphrodites respectively of the cultivated varieties, as it has been shown that the cultivated hermaphrodites have probably been derived from partially fertile wild male types (7). In the strawberry, as in the grape, it may be assumed that the factor for femaleness (*F*) carries linked with it the suppressed male factor, and that the functional male factor is linked with a factor for femaleness which is suppressed to the extent that only somatic structures are developed and the pistils are sterile. In the case of the hermaphroditic strawberries, the female condition is not suppressed, and the pistils are therefore fertile. The symbol *H* then represents the linked factors *F* and *M*.

The seedlings studied fall into five groups based on parentage. These are as follows: Hermaphrodite × self, hermaphrodite × hermaphrodite, male × male, female × hermaphrodite, and female × male. The various combinations will be considered separately.

The term *female* will be used in referring to pistillate plants bearing abortive anthers; *male* in referring to the somatic hermaphrodites which generally bear sterile pistils, and the term *hermaphrodite* in referring to those perfect-flowered forms which are fertile with regard to both pistils and pollen.

Hermaphrodite × Self

Table 1 gives the results of self-pollinating hermaphroditic varieties of strawberry. A total of 352 hermaphrodites, or somatic hermaphrodites, and no pistillates were produced when 11 distinct clones were selfed.

Table 1. *Results of Self-pollinating Perfect-flowered Varieties of Strawberry*

Lot No.	Variety or Seedling Selfed	Hermaphrodites	Sex of Progeny	
			Pistillates	Staminates
1/15	778	68		
1/16	F_1 of (778 × self)	2		
32/16	F_1 of (778 × self)	18		
57/16	F_1 of (*F. virg.* ♀ × 778)	46		
6/15	South Dakota	19		
3/16 and 40/16	Dunlap	72		
4/16 and 26/16	Minn. no. 3	59		
63/16	Fendell	3		
	Prolific	2		
54/16	F_1 of (Glenville × self)	5		
15/16	Glenville	28		27
	Total	322		30

In view of the number of different parent plants used, the results go to prove that hermaphrodites do not carry the factor for femaleness and are therefore homozygous for the hermaphrodite determiners. These results are in keeping with the condition found in the hermaphroditic wild species *F. americana*, which must be considered to be homozygous for the hermaphrodite determiners. They are also in keeping with results of strawberry breeders who never expect pistillate varieties to result when only hermaphrodites are used as parents.

Although all these seedlings were hermaphrodites as far as somatic condition is concerned, there were distinct differences in the fertility of some of them. Lot 32/16, an F_1 of 778 × self, produced 18 fertile plants and one which was sterile. Sterility in this one may have been the result of weakness, due to self-pollination or to some other cause.

An hermaphroditic F_1 progeny of *F. virginiana* ♀ × 778 ☿ (lot 57/16)

when selfed produced 46 hermaphrodites of varying degrees of fertility, and 2 males which were completely sterile. As will be shown later, the pistillate strawberry plants appear to be heterozygous for sex, and therefore the female grandparent *F. virginiana* would be of the constitution *FM* and when crossed with 778 (*HH*) should give 1 female (*FH*) to 1 hermaphrodite (*HM*). *HM* was the supposed constitution of the hermaphrodite selfed in lot 57-16. The expectation from it when selfed would be 1 *HH* : 2 *HM* : 1 *MM*. There were produced 46 hermaphrodites and 2 males, one of which was very weak. The hermaphrodites varied in fertility. Five were completely fertile in all flowers; 26 set all but quaternary flowers; 14 were fertile in primary and secondary flowers while the tertiaries were either sterile or produced nubbins and the quaternaries were sterile; 1 produced only nubbins and was practically a male. Although it is difficult to classify each of these with respect to its genetic constitution, it seems apparent that all the expected classes are represented; the *HH* being those completely or nearly completely fertile, the *MM* those completely sterile, and the *HM* group those showing intermediate degrees of fertility. These results are significant of the constitution of the wild female, as they show conclusively that the tendency toward sterility of a dioecious species may be transmitted by the wild female, which is fertile, to hermaphroditic progeny; whereas its female progeny are always completely fertile. A probable explanation for the variations in fertility of the *HM* individuals will be discussed later.

Lot 15-16, Glenville × self, although conforming to expectation as far as somatic characters are concerned, was strikingly different as to fertility from the other selfed hermaphrodites. This variety was mentioned in a previous paper (7) as being practically sterile when grown under field conditions although it produced blossoms profusely. When grown in a bench in the greenhouse it exhibited the same sterility in the first crop of blossoms, but when potted and kept in the house until a second crop of blossoms was produced it exhibited a fairly high degree of fertility. It is evident that environment plays an important part in the fertility of certain clones. This variety (15-16) when selfed produced 55 seedlings, 27 of which were completely sterile under field conditions while 28 produced some fruit. Of these, 11 exhibited a fairly high degree of fertility under field conditions, while the remainder set only an occasional flower and the berries produced were often nubbins. One seedling, which produced an occasional berry when grown in the greenhouse, was self pollinated (54-16). It produced 5 seedlings all of which were sterile to a high degree, setting only an occasional achene on some of the primary flowers. It appears from the results obtained that Glenville carries an hermaphrodite factor which is fertile and another which is practically sterile. Under certain cultural conditions, the first of these factors is dominant and fruit results, while under other conditions the other is dominant and the plant is sterile.

Hermaphrodite × Hermaphrodite

The cross hermaphrodite × hermaphrodite should give the same results as to sex types as hermaphrodite × self. The results of 11 such crosses are given in table 2.

Table 2. *Results of Crossing Perfect-flowered Varieties of Strawberry*

Lot No.	Varieties Crossed	Sex of F_1 Seedlings		
		Hermaphrodite	Pistillate	Staminate
14/16	Fendell × Dunlap	25		
23/16	Fendell × Minn. no. 3	4		
71/16	(F_1 of S. Dakota × self) × (F_1 of 1017 × Progressive)	32		
24/16	(F_1 of 778 × self) × Minn. no. 3	2		
34/16	(F_1 of 778 × self) × Glenville	20		
12/16	Dunlap × Glenville	8		
47/16	(F_1 of *F. virg.* ♀ × 778) × Glenville	14		
47/16	(F_1 of *F. virg.* ♀ × 778) × Glenville	4		
48/16	Minn. no. 3 × Glenville	28		
53/16	(F_1 of 778 × self)* × Glenville	67		
18/16	(F_1 of Glenville × self) × Minn. no. 3	4		
	Total	208		

* Stamens of intermediate type between staminodes and perfect stamens.

These results are similar to those obtained by selfing hermaphrodites, with the exception of one cross which gave apparently a 1 : 1 ratio of hermaphrodites and pistillates. These were the progeny of a cross in which an hermaphroditic F_1 seedling of *F. virginiana* ♀ × 778 ☿ was used as the female parent and Glenville as the male parent. According to our hypothesis, the female parent (hermaphrodite) derived from *F. virginiana* ♀ × 778 should be of the constitution *MH*, the *M* representing the male determiner carried by a wild female. Glenville we may consider to be *HH*. From such a cross all the progeny should be hermaphrodites at least as far as somatic condition is concerned. It seems probable that this was the case in view of the stamen condition found in a portion of the flowers in other combinations in which 778 was used. When 778 was selfed it produced only hermaphrodites. Some of these developed stamens which produced only a small amount of pollen in some flowers while the stamens of other flowers were practically identical with the staminodes of pistillate plants. One of these clones was used as the female parent in a cross with Glenville (table 2, 53/16). This cross produced 67 hermaphrodites. Forty-seven of these developed normal stamens in all the flowers. Twenty showed a decided tendency toward the production of staminodes similar to those found in pistillate flowers or of the intermediate type found in their female (hermaphroditic) parent. Eleven of the twenty bore flowers which pro-

duced many staminodes and intermediate stamens and a few normal ones, while the other 9 produced many flowers which could not be distinguished from those borne on pistillate plants. Only an occasional perfect flower appeared to indicate the true sex condition of the plants. I believe that the five apparently pistillate seedlings resulting from the cross 47/16 were of this type, but were not observed at a time when perfect flowers were present.

Of two hermaphrodite × hermaphrodite crosses made by Mr. Charles Haralson, one, 1017 × Francis, resulted in 43 hermaphrodites, all very fertile, and no females; the other, 1017 × Progressive, resulted in 1,105 hermaphrodites and 33 females. Here, again, the writer believes that these were not true females but were hermaphrodites observed at a time when no functional stamens were present. This view is strengthened by the fact that in this lot of 1,138 seedlings, 433 hermaphrodites produced some female flowers. In some of these the primaries only were pistillate, while in other clones only an occasional perfect flower was produced (7, Pl. 35). An occasional pistillate flower is quite common in many of the hermaphroditic varieties, especially among the primary flowers produced early in the spring. They are always fertile to a high degree, and produce the largest berries borne on the cluster. Wild males of *F. virginiana* may also produce an occasional pistillate primary flower which is generally fertile. A more extensive study of such types as these may throw light on the origin of dioeciousness in the genus Fragaria.

Staminate × Staminate

The cross staminate × staminate should give results identical, so far as somatic flower types are concerned, with the combinations thus far considered. Only a single combination of this type was studied. The female parent was a staminate plant of *F. virginiana* which produced a single pistillate primary flower. This, when pollinated with pollen from a staminate *F. virginiana*, set a few seeds. These produced 4 plants, all of which were somatic hermaphrodites or males. The cross was truly staminate × staminate rather than hermaphrodite × staminate, as the plant used as the female would always be considered a pure staminate in nature. Thus far we have obtained a total of 1,714 hermaphrodites or somatic hermaphrodites to a possible 38 pistillates when hermaphrodites were pollinated from hermaphrodites in 25 different combinations. In view of the explanation given of the probable sex condition of the 38 pistillates, the results leave little doubt that hermaphrodites or somatic hermaphrodites carry only the hermaphrodite and male determiners and do not contain the determiner for femaleness. The strawberry is then in direct contrast to the condition found in Lychnis and Vitis, in both of which the hermaphrodites have been shown to be heterozygous for the sex determiners.

Pistillate × Hermaphrodite

Table 3. *Results of Crossing Pistillate with Perfect-flowered Varieties of Strawberry*

Lot No.	Varieties Crossed	Sex of F_1 Seedlings		
		Hermaphrodite	Pistillate	Staminate
28/16.	(F₁ of Crescent × ?)	21		
	× Minn. no. 3		16	
40/16.	Enormous × Dunlap*			
	Bederwood × Glenville* (7 plants)	14	11	
40/16.	Enormous × Dunlap	5	7	
40/16.	Enormous × Dunlap*			
	5/15 67-2 ♀ × Glenville*	13	14	
66/16.	Columbia × 5/15 70-4	26	27	
5/15.	*F. virginiana* × 778	18	18	
33/16.	5/15 62-5 × Glenville	27	37	
45/16.	5/15 64-2 × Glenville	32	28	
49/16.	Bederwood × Glenville	26	28	
65/16.	Columbus × Glenville	9	10	
56/16	5/15 × Glenville	8	5	
	Total	199	201	

* Plants mixed by accident.

If we accept the chromosome theory of inheritance as applied to sex in the strawberry, we must conclude that the females are heterozygous for the determiners *H* or *M* and *F*, depending on whether cultivated varieties or wild pistillates are concerned, in view of the fact that the male plants and the hermaphrodites have both been shown to be homozygous for sex determiners.

The results of crosses in which pistillates were used as the female parent and various hermaphrodites as the male parent are given in table 3. There is a definite 1 : 1 segregation of the 400 seedlings into 199 hermaphrodites and 201 pistillates.

In four crosses of pistillate × hermaphrodite made by Mr. Charles Haralson and recorded by the writer, the results shown in table 4 were obtained.

Table 4. *Results of Crossing Pistillate with Perfect-flowered Varieties of Strawberry*

	Pistillate	Hermaphrodite
Seedling 1020 ♀ × Progressive	31	41
Seedling 1020 ♀ × Seedling 907	16	9
Meteor ♀ × Seedling 907	28	22
Productive ♀ × Seedling 1017	4	5
Total	79	77

These results leave little doubt that the pistillate plants of Fragaria are heterozygous with respect to the sex-determining factors. The results of the writer in crossing pistillates by hermaphrodites are in keeping with the

results obtained by Richardson (2), who obtained a total of 203 ♀ and 173 ♂ or ☿ in making similar crosses.

Sex inheritance, in all these crosses, conforms to the theory of a heterozygous condition of the females, the female condition being completely dominant, with one possible exception. This was the cross 45/16 in which 5/15 64–2, a pistillate, derived from the cross 5/15 (table 3) was used as the female parent and Glenville as the male parent. This cross resulted in 32 hermaphrodites and 28 females and approximated the expected 1 : 1 ratio. The fertility of the seedlings, however, was not what was expected. Fifteen of the females set all flowers except an occasional late one but produced only a few achenes per berry, and as a consequence all berries were nubbins. The remaining 13 females were completely fertile except for an occasional late flower. Twelve of the hermaphrodites set some fruit and in some cases nearly as many flowers as the females, but all the berries were likewise nubbins. Eleven hermaphrodites set nearly all flowers and the berries were perfect; 9 others set one half or less of the flowers perfectly. The remainder were males. The expectation from this cross was 1 fertile female to 1 hermaphrodite varying in degree of fertility. In other words, the females should all have been completely fertile and the hermaphrodites either fertile or partially sterile depending on whether the *H* from the female parent united with the more fertile determiner of Glenville or with the one which has been shown to be practically sterile; and on the extent to which the *H* determiner from the female parent is dominant over the sterile *H*. Instead of the expected ratio, 1 normal female to 1 practically sterile female (nubbins) to 1 fertile hermaphrodite to 1 partially fertile hermaphrodite was obtained. This and one other are the only cases observed in which females have not been nearly completely fertile. There has evidently been a decided change in the female determiner in this single instance. For an explanation of this peculiar condition it seems we must go back beyond the two immediate parents. The male parent Glenville is probably not the cause, as, when crossed with other females, it has not produced similar results. The female parent was the offspring of a wild female × hermaphrodite 778. Two other F_1 females of this cross acted according to expectation when crossed with Glenville (56/16 and 33/16, table 3). It seems, therefore, that we are forced to assume that a change took place, probably during the reduction division, in the wild female grandparent. A crossing over in the female and male chromosomes between the female determiner and the suppressed male determiner and *vice versa* in the male chromosome would give a chromosome in which both sexes are suppressed: (*mf* or *h*). An egg containing such a chromosome if fertilized by a male gamete from 778 carrying an hermaphrodite determiner would produce an individual having one determiner for femaleness and one for maleness, these two being linked (*H*). The constitution of this individual would then be *Hh*. We have already seen that a single dose of femaleness is

sufficient to produce fertile pistils (wild female) while one dose of maleness is not sufficient to produce functional stamens. This plant would then be a female. The only records on this individual, which was grown in the greenhouse, stated that it was female and had berries strikingly like the berries borne on the original wild female. The berries borne on its sibs were all much of a type and more intermediate between its parents, although most F_1 plants of *F. virginiana*, when a pistillate is used as a female parent, resemble the wild to quite a degree. If we are correct in the assumed constitution of this female, and if it is crossed with Glenville, the expected combinations would be as follows: hH (female) $\times$ HH^1 (Glenville in which H is a normal hermaphrodite and H^1 is a weak hermaphrodite or male) = 1 hH (a fertile female) : 1 hH^1 (a female sterile or producing only nubbins): 1 HH (a fertile hermaphrodite) : 1 HH^1 (a partially fertile or sterile hermaphrodite of the Glenville type). These are in fact the types which were produced. A similar explanation might be given for the origin of the completely sterile wild clones of *F. virginiana* which are occasionally found in which neither the stamens nor the pistils are functional. It is not maintained that this is the correct explanation of the results obtained. It is based on the assumption that the male and female determiners in an hermaphroditic chromosome are separate and distinct, and that, as a consequence, crossovers might occur between them. The four types of plants were very distinct, and the numbers were so large as to indicate a genetic condition different from any previously studied.[2]

Although the results thus far given all point to the hermaphrodites being homozygous for sex determiners, and the pistillates heterozygous, they are not absolutely conclusive, since the progeny of selfed females have not yet been studied. This I believe will be impossible in the strawberry, as I have never found a stamen on any of the cultivated pistillate varieties or on wild pistillate clones which showed any signs of producing pollen. If, however, we can transfer the sterile male condition of the wild *F. virginiana* to progeny through the use of a wild *F. virginiana* female with fertile hermaphrodites, it would seem that we have proved its heterozygous condition for F and M. This seems to have been done in the cross 5/15 (table 3) in which a wild *F. virginiana* ♀ was the pistillate parent and the very fertile hermaphrodite 778 was the male parent. This cross produced 18 pistillates, all fertile to a high degree, and 18 hermaphrodites. Nine of the hermaphrodites were completely fertile, 4 were fertile except for an occasional tertiary or quaternary flower, while 5 set only an occasional fruit. On the theory that pistillates are heterozygous, the genetic constitution of the wild female would be FM and that of the hermaphrodite HH^1. The female progeny of this cross would then all be FH or FH^1 and would be completely fertile. The constitution of the hermaphroditic progeny would

[2] The other case in which a female was nearly sterile resulted from the cross 65/16, Columbus $\times$ Glenville, which produced 9 hermaphrodites and 11 females, 10 of which were completely fertile while one produced only nubbins.

be HM and $H'M$, depending on which of the H chromosomes of 778 combined with the M. What should be the sex of these new genotypes? They should be hermaphrodites as far as somatic condition is concerned and should be completely fertile, partially sterile, or sterile, depending on the extent to which their sex is determined by the hermaphrodite or male determiners and on whether the two hermaphrodite determiners have an equal or an unequal potential fertility. The hermaphroditic progeny were of two classes consisting of 9 completely fertile clones and 9 partially sterile, as was previously mentioned. These results would seem to indicate that the two H factors of 778 differed slightly in potential fertility and, when in combination with the same male factor, resulted in different degrees of fertility. This does not necessarily follow, however, as it will be shown later that a single H factor of a female plant, when united with the two male factors of a wild male, produced progeny showing the same variations in fertility. The result, when one of the 5/15 (*F. virginiana* ♀ × 778) hermaphrodites was selfed, was reported in table 1, 57/16, and was analyzed in the following discussion. It was there shown that pure males, completely fertile hermaphrodites, and intermediates could be produced from one of these HM hermaphrodites. These results prove that the wild females carry the factor for maleness, femaleness being completely dominant over it, and that the factor for maleness can be transferred through the wild female to hermaphroditic progeny, and that, when in combination with an hermaphrodite factor, either one may act as a dominant, producing complete fertility or sterility, or both may show partial dominance resulting in partial fertility.[3]

The question as to whether one hermaphrodite of the constitution HM may be fertile and another partially sterile, or whether one H factor may be potentially more dominant than another, is extremely important from an economic as well as from a scientific standpoint. The case of Glenville, previously mentioned, in which the sex varied from male to hermaphrodite under different cultural conditions, and which produced, when selfed, both fertile and sterile seedlings, is evidence that one of the sex factors may be dominant at one time and the other dominant under other conditions. Further studies on the relative dominance and potential fertility of the H factors in the cultivated strawberry may throw considerable light on the reasons why certain varieties fruit heavily under some conditions and produce an inferior grade of irregularly shaped fruit under other conditions.

Pistillate × Staminate

The cross pistillate × staminate should give the same results as pistillate × hermaphrodite as far as somatic appearances are concerned. The

[3] Both the H and the M factors must be considered to be recessives, as both are recessive to the F factor. This seems to be true in spite of the fact that the H factor appears to carry functional F and M factors linked.

progeny of only a single cross of this type has been studied. The female parent used was a pistillate derived from crossing a wild pistillate *F. virginiana* (*FM*) by the hermaphrodite 778 (*HH*) (5/15, table 3). All pistillates from such a cross should have the constitution *FH*. This female was crossed with a wild *F. virginiana* staminate. Twenty F_1 seedlings were obtained. Eleven of these were pistillate and 9 hermaphrodites or somatic hermaphrodites, thus giving a close approximation to the expected 1 : 1 ratio. The pistillates were all very fertile. Of the 9 hermaphrodites obtained, 3 set no fruit while 6 set fruit in varying amounts, from only a nubbin on the primary flower with the others sterile, to some which set fruit on flowers of all degrees but with a portion of the tertiaries and quaternaries sterile. If the 9 hermaphrodites are divided into two groups with regard to whether sterile flowers or fertile flowers predominate, there will be 6 classed as sterile and 3 as fertile. These plants are all of the constitution *MH*, the *H* factors all being identical and the *M* factors being derived from the wild male parent. Thus the male factor of the wild female was replaced by an hermaphrodite factor in the female parent of this cross, and maleness was introduced into the progeny by a wild male (*MM*). The results of this cross are similar with respect to fertility of the hermaphrodites to those obtained from cross 5/15 in which a wild female (*FM*) was crossed with a cultivated hermaphrodite (*HH*). It is thus seen that maleness may be introduced into otherwise fertile strains of strawberry by the use of either the wild females or the wild males. Even though the flower types with respect to fertility of the hermaphrodites are not always clear-cut, these results are in accord with the chromosome theory of sex inheritance. It would seem that the outward expression of a given sex determiner may be influenced by the sex determiner with which it is associated and also by the autosomes associated with the sex chromosomes in the nucleus.

Discussion

The Sex Determiners

In the above presentation of data, and in the conclusions which have been drawn from them, it has been assumed that the determiners for sex are definite factors and that they are carried in a definite pair of sex chromosomes. We must assume that the various sex conditions which appear have been derived originally from an hermaphroditic condition in which the determiners for the two sexes are linked in each of a pair of chromosomes. The various sex types which appear in either the grape or the strawberry, or, in fact, in any of the flowering plants which are dioecious and show a variety of sex types intermediate between staminate and pistillate, we assume to have been derived by suppression, either partial or complete, of one of the determiners for sex in the sex chromosomes, leaving the other factor functional (5). It can hardly be assumed that one or the other sex

determiner is ever completely eliminated, since abortive organs, either stamens or pistils, are often present in flowers of the opposite sex in supposedly strictly dioecious forms, and occasionally even perfect reproductive organs of the opposite sex are found in such "strictly" dioecious forms as *Acer Negundo.*

In the strawberry the assumption of partial suppression of the female determiner is necessary in both the sex chromosomes of male plants of *F. virginiana*. Femaleness is still present and functional to the extent of producing apparently normal pistils, which, however, prove to be sterile. In the hermaphrodites of the cultivated varieties (derived from dioecious wild species) suppression of femaleness is very slight but is still present to a degree in many cases, as shown by the decreased fertility of such forms when compared with pistillate varieties (7). In some hermaphrodites there is apparently no suppression of femaleness, since they may be completely fertile. The pistillate plants of *F. virginiana* we could assume to contain two sex chromosomes which are different. One of these would be identical with the two found in wild staminates, *i.e.*, male (+ female suppressed), and the other bearing the normal female determiner linked with a suppressed male determiner. Cultivated pistillate varieties would then be of the constitution female (+ suppressed male), and hermaphrodite. In other words, we should assume that a single dose of femaleness, if carried as an *F* factor, is sufficient to produce fertile pistils in the pistillate plants, while two doses of maleness are necessary for the production of functional stamens. If only a single dose is present, staminodes are produced. On the other hand, if there is present a single dose of femaleness linked with a normal male determiner, thus forming the recessive *H* factor, it may or may not be sufficient to produce functional pistils.

An interpretation of the data presented on the basis of these assumed chromosome conditions leaves little doubt that the males and hermaphrodites are homozygous for sex chromosomes bearing functional male determiners. The females, on the other hand, must be assumed to possess one sex chromosome carrying a male or hermaphrodite determiner, and another chromosome carrying the female determiner. In other words, we have a condition similar to that existing in pigeons and cultivated fowl in which the males are apparently homozygous and the females heterozygous for the sex determiners. The condition is opposite to that which has been found to exist in the females of Bryonia, Lychnis, sweet pea, and Vitis. In view of the fact that either males or females may be heterozygous in the animal kingdom, it is not surprising that both types should be found in plants.

The writer has not attempted to give a review of the literature on sex inheritance and sex determination in plants, since this has recently been done rather comprehensively by Yampolsky (8, 9, 10).

Although a factorial basis for sex determination is rather generally admitted to be correct for explaining inheritance of sex in animals, Yampol-

sky and others are not inclined to believe that it will also explain the conditions found in plants. This is due primarily to the fact that an individual may during its life or during the flowering of a single cluster appear to change its sex. In other words, there may be "a periodic alternation of sex." Yampolsky (10, p. 99) holds that "a factorial hypothesis for sex can not explain these results." In his work on sex intergrades and sex inheritance he worked primarily with *Mercurialis annua*. The material is unfitted for a study which will readily produce accurate results because of the mechanical difficulties encountered in making the crosses necessary for a proper interpretation of the genetic constitution of the several plant types. His conclusion that males when selfed tend to produce males or plants predominantly male, and that females selfed tend to produce females or predominantly females was based on the results of selfing a large number of flowers on female plants, of crossing females with males (which produced a 1 : 1 ratio of males and females), and of selfing female flowers on male plants. His results obtained by selfing female plants would seem to show conclusively that the females are homozygous for sex determiners, if we attempt to explain the results on the factorial basis. Females × males produced a 1 : 1 ratio of females and males, as would be expected, and the results suggest, in view of the results obtained from selfed females, that the males are heterozygous for the sex determiners. Although self-pollinated males produced only males, the statement (8, p. 434) that "the selfed males of *Mercurialis annua* may be said to record their own gametic constitution" is not based on sufficient evidence to warrant discarding the chromosome hypothesis of sex inheritance in plants. Yampolsky does not take into consideration the possible effect of lethal factors on sex ratios. There is abundant evidence from his results to indicate that the sex ratios of the progeny of his selfed males may have been influenced by lethal factors which inhibited the normal development of the embryos or which later affected germination either by weakening it or by destroying it completely. From a total of 156 female flowers on one lot of male plants he obtained 283 seeds. Of these, 31 were immature. He explained immaturity as the result of one seed developing faster than the other in two-seeded ovaries, so that in gathering the seeds the immature one is likely to be gathered with the mature one. Of the 283 seeds obtained from this lot of male plants, 219 were sown. Of this number 75 *seedlings only developed*. These were all male plants. It is obvious that something interfered with the normal development of the other seeds. Certain ones developed slowly; others germinated weakly and then died; while others did not germinate at all. Horticulturists are generally aware of the fact that slightly immature seeds germinate nearly as well as, and often better than, mature ones. There is a question as to whether immaturity can be correctly assigned as the cause of failure of any of the seeds to germinate. Yampolsky (8, p. 432) has used the work of Shull (4) on irregular sex ratios in *Lychnis*

dioica as an argument against a Mendelian explanation of sex inheritance, stating that "the Mendelian hypothesis of sex in itself can not account for the preponderance of one sex to the exclusion of the other." The writer (7, p. 663), however, has shown that wherever in Shull's work (3, 4) irregular sex ratios were obtained, they were the result of partial or complete elimination of the male (and to a slight extent of the female) gametes which carried the mutant factor "narrow leaf" linked with femaleness (or with hermaphroditeness in the case of *Melandrium album*). This explanation was borne out by the presence of large amounts of abortive pollen in the narrow-leaved Lychnis male. Dorsey (1), working with plums, has expanded the idea of gametic elimination due to unfavorable genetic combinations to include the elimination of embryos during various stages of their development because of unfavorable genetic combinations and has given considerable evidence in support of this explanation as the cause of dropping of plums through the summer and of poor germination of apparently normal seeds from certain crosses.

It appears from Yampolsky's results with seed of selfed males that immaturity and lack of germination in the seeds from selfed males may be due to unfavorable genetic combinations which allowed development of certain embryos to proceed only to a certain point, when growth ceased. In his table 6 (8, p. 422) are reported a total of 156 flowers which produced 283 seeds on male plants. Besides these there were produced on the same plants "approximately 90 other female flowers . . . which failed to develop seeds and dropped off." A total of 492 seeds might then have been expected if all had set and developed normally; 283 were actually produced, and of these, 31 were immature, 5 germinated weakly, 139 did not germinate, and 26 were not planted. Certainly there is evidence of elimination in these results, and they can not therefore be taken as indicating that the genetic condition of the males of Mercurialis is pure male and not heterozygous for male and female determiners, as would be indicated by the results of crossing females with males. All possible genetic combinations should be thoroughly tested before conclusions as to the genetic conditions with respect to sex may be safely arrived at. The species in question makes such a study extremely difficult.

In Fragaria, as in many other forms, the sex of all the flowers of any given plant is not necessarily the same. It is quite common in the perfect-flowered varieties for practically all the earliest flowers to be female. In some clones this condition has been exaggerated to a point at which only a few flowers produce stamens while the remainder are female. Male flowers on hermaphroditic varieties are common, and are practically always limited to the last flowers of a cluster to bloom. Female flowers may be found in the primary position of the inflorescence of wild males, and in such cases generally set fruit. Completely sterile flowers are not uncommon among the later flowers of a female inflorescence.

In general, in Fragaria there appears to be a tendency towards femaleness in the earliest flowers which bloom; while the later flowers have a tendency towards maleness in the hermaphroditic varieties. A similar tendency with regard to increasing pistil sterility in the later blossoms is apparent, but to a lesser degree, in the cultivated females. The females of dioecious wild forms and all the plants of the wild hermaphroditic forms exhibit an extremely high degree of pistil fertility extending through all the flowers of a cluster. It is seldom that irregular berries are seen in wild forms, even under the most adverse conditions.

The writer does not believe that the presence of other sex types of flowers on a plant, which is predominantly of one sex, necessarily means that sex is not determined by specific factors or that such a condition is an argument against a Mendelian interpretation of sex in plants.

If it is assumed that dioecious forms have been derived from hermaphroditic forms, and if we accept, for the present, the theory that in hermaphrodites the two sex determiners are linked in one chromosome, then the production of males and females in such forms as Fragaria and Vitis, where the opposite organs are still present but not functional, must have come about by the partial suppression of one or the other sex condition in the chromosomes but not by its complete loss. Thus, in the female plant of *F. virginiana* two sex chromosomes would be present: one which carries the functional factor for femaleness (which is sufficient to produce functional pistils), and another which carries the functional factor for stamen production, but which, in the absence of another similar functional factor, is unable to produce functional stamens. Each chromosome, however, would carry the opposite factor suppressed. The males would then contain two like chromosomes, each of which carries a functional determiner for stamen production and a suppressed determiner for femaleness. It appears that no other assumption will meet the actual conditions. It is apparent from the facts previously given that the sex condition may be varied by varying the conditions under which the plants are produced. This may result by changing either the outer environmental factors of the plant or the internal factors of nutrition (?) due to the position of the flowers on the inflorescence. Such changes have been observed. It is not a far step, then, to assume that in certain parts of a plant conditions are set up which have a tendency to decrease the suppression of factors which are already present and to allow flowers or flowering parts of the opposite sex to be produced on a given plant. The determiners for sex in plants may be specific entities, but still their full or partial expression may depend upon the immediate conditions surrounding them at the time of flower production.

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